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The Journal of Laboratory and Clinical Medicine

VOL. XIII ST. LOUIS, MO., FEBRUARY, 1928 NO. 5

CLINICAL AND EXPERIMENTAL

AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

PATHOLOGY OF INTESTINAL TUBERCULOSIS*†

BY ALFRED BLUMBERG, M.D., PH.D., PHARM.D., OTEEN, NORTH CAROLINA

INTESTINAL tuberculosis may be a primary condition in children. It occasionally happens that intestinal manifestations make themselves known in adults before the pulmonary tuberculous infection is recognized. Whether such manifestations represent primary intestinal tuberculosis or are merely the result of tuberculotoxic influences is still a matter to be proved. As a rule intestinal tuberculosis in the adult is a secondary manifestation of far advanced active tuberculous infection of the pulmonary tissue. The condition is of frequent occurrence. In far advanced active cases it may be present in from 70 to 80 per cent. In moderately advanced active cases it may be present in from 14 to 18 per cent, while in the early cases it may exist in from 5 to 8 per cent.

It is assumed that intestinal tuberculosis is the result of the successful deposit and multiplication of the tubercle bacillus within the intestinal wall. The organism is carried to this place from an existing focus either by means of the lymphatics or the blood vessels, or, perhaps, most often through the alimentary canal by means of swallowed sputum. In children, food containing tubercle bacilli may also be responsible. Why is it that intestinal tuberculosis is more frequent with the far advanced active patient than it is with the far advanced inactive patient or in moderately advanced active cases? Tendeloo[1] is of the opinion that the number of bacilli introduced into the alimentary canal is a factor in the possible creation of intestinal lesions. He believes that tuberculous tissue changes appear only when the "poisonous potency" of the organisms has reached a given optimum, as a result of which the tissue becomes susceptible, and such changes result. This assumption

*Read before the Sixth Annual Convention of the American Society of Clinical Pathologists in Washington, D. C., May 13, 14 and 16, 1927.

†Permission for the publication of this article was obtained from the Director of the U. S. Veterans' Bureau, Washington, D. C.

does not fully explain the question raised. If we consider that a sputum containing but two or three organisms per microscopic field will deliver many thousands of tubercle bacilli per c.c., we realize that many millions of organisms will pass over the intestinal mucosa during the twenty-four hours of the day. We also realize that introduction into the alimentary canal of such organisms will not be limited to a single day, but to the passing of such sputum over the mucosa for many months without the existence of intestinal lesions. Tendeloo defines "poisonous potency" as: Virulence multiplied by number of bacilli. This formula, however, is insufficient to explain successfully the cause of tuberculous extension in other parts of the body from the primary focus.

As has been mentioned, a constant supply of material rich in tubercle bacilli fails for a time to produce lesions in cases of moderately advanced active tuberculosis. It is difficult to believe that such patients fail to swallow some of their sputum during the course of the disease. Still many of these patients offer not the slightest evidence of intestinal infection. In fact, upon postmortem examination a certain number of far advanced cases show no gross tuberculous lesions in the intestines. Whether the intestinal wall in these cases is free from tubercle bacilli cannot be stated, for it may be that in spite of the absence of gross lesions some bacilli may have passed the mucosa, and that the failure to find such organisms upon histologic examination may be due to the scarcity of the bacilli and to the probability that a part was examined in which no organisms had been deposited.

It is not denied that ingestion of tubercle bacilli gives rise to intestinal tuberculosis; in fact, a wide literature tends to confirm this (for literature see Baumgarten, Bolinger, Cornett, McFadden, Kovacs, Rabinovich).[1] The conclusions drawn by the various writers are that the susceptibility is greater in younger animals; that in a number of cases tuberculous infection macroscopically confined itself merely to the mesenteric glands; that it was possible to find tubercle bacilli in the blood, lungs, liver, and mesenteric glands of the animal from seven to twenty-two hours after their having been fed with the organisms, and that the lymph follicles of the intestines are very prone to be damaged by the tubercle bacilli.

Tendeloo thinks that certain factors are responsible for the favorable deposit of the organisms in the intestines. These factors are as follows: An empty stomach or empty intestine will allow the passing through the mucosa of the bacillus with greater readiness than an alimentary canal which is not empty. Certain types of vehicles may serve the tubercle bacillus, as for instance milk, which carries the organisms and allows their deposition upon the intestines with great readiness.

I do not believe that the causes mentioned explain successfully why infection occurs with some patients and not with others. From our clinical observations and autopsy material examined, we learned that most of the patients have had some functional disturbances. It may be assumed that changes of such type predispose to successful tuberculous invasion which would not occur in a perfectly normal structure. Patients who have a chronic gastritis, who show atrophy in some parts of the mucosa of the small

intestine, or patients with extensive or localized stasis in the small intestine and such who have complained of meteorism as a result of the stasis, are by far more likely to be the victims of this condition. The circulatory disturbance which gives rise to stasis results ultimately in meteorism, which again leads to increased stasis. This vicious circle prepares the intestine for the reception and the development of the organism. These changes are perhaps the result of tuberculotoxic influences which gradually alter the normal function of the cells and are responsible for the complaints of the patient. There are strains of tubercle bacilli which are able to produce tuberculotoxic products in greater quantity than do others. The individual cells of the tissues possess biochemic substances, most likely belonging to a class of fatty alcohols, which serve to protect against toxic influences. In time, however, the cells gradually become deprived of such protection. The sugar content of the cell is also reduced, and an opportunity is given to the organism to invade this exhausted portion of the tissues.

I believe *that the degree of virulence of the organism lies in the extent of its ability to exhaust biochemic materials from the cell, without which material, the cellular metabolism is impaired; the function to neutralize toxic substances is weakened, and the reproducing ability of the cell is diminished.* The optimum expression of such virulence allows successful invasion. Many organisms possessing but a small amount of such virulence will give rise to little, or no, activity unless they are numerically increased to that extent where the necessary quantity of toxic substance has accumulated to inactivate cellular function. On the other hand, a comparatively few organisms may possess the ability of producing extensive tuberculotoxic substances.

Certain types of cells have a great ability to maintain their biochemic contents; others are less able to withstand outside attacks. It is quite well known that one of the easiest damaged tissues is the epithelium, which lines mucous surfaces. That such epithelium is damaged in the intestines before successful invasion occurs is assumed. Of course, no epithelial destruction is necessary when the bacilli are distributed by either lymph or blood stream. That the blood stream is, at least in certain cases, responsible for the occurrence of lesions in the intestines has been shown where general hematogenous dissemination gave rise to the presence of miliary tubercles in the intestines. Such miliary dissemination in the intestines has occurred in 4.3 per cent of our cases. That the blood stream does carry organisms has been shown repeatedly, and the probability is that organisms carried by the blood stream at one time or another will be deposited and lead to consequent tissue changes.

The lymph stream also carries organisms. The specimen is an example of a condition which is met with fairly often. It occurred in 8 per cent of our cases. The specimen shows tubercles which, according to MacCallum, are actually formed in the lymph channels in a manner to block the lumen. The lymphatics become conspicuous, and as a result of distention by clear fluid, or opaque chyle, the beaded arrangement is formed, the obstruction occurring at intervals. It is assumed that these lesions represent the path over which the organisms traveled before they reached the mesenteric lymph nod-

ule which is the seat of the secondary tuberculous deposit. Whether the same appearance of the lymphatics is ever the result of the extension by retrograde process from the lymph nodule to the intestine is not known, although opinions exist that the upstream course of the tubercle bacilli is a possibility. Kaufmann[2] states that "tuberculous infection of the intestine may be the result of extension in a retrograde manner from the mesenteric glands to the intestinal wall. E. Edens describes and the author (Kaufmann) had also seen it."

Once successfully deposited in the intestinal wall, the organism after multiplication will soon, as a result of its activity, show gross evidence of its existence. Perhaps the earliest visible manifestation is a papillary elevation over the mucosa, conspicuous more by its pallor than by its size and elevation. This lesion is from one to one and one-half mm. in diameter, elevated, domed, having a broad base and with no depression in its dome at this stage. Soon the dome flattens out, and the lesion becomes circular or oval in outline, and there may be a faint suggestion of a central dimple which in time becomes definitely visible. The formerly pale lesion is now of dark or reddish-brown color, although a fine pale line may still be noticeable at the junction of its base with the mucosa. As a rule, other similar lesions have developed during the same period. The lesions have a tendency to coalesce, and the approximated walls will by and by disappear. The manner of fusion of these lesions will act to a great extent in deciding whether the resulting ulcer will take a course along the intestinal folds or across them. The most frequent ulcer met with is the one encircling the mucosa of the intestine. The longitudinal was present in 3 per cent of our cases. The irregularity in the distribution of the small lesions will, after fusion, cause to a certain extent, the lace-like outline seen in the larger ulcer, although progressive necrosis beneath the elevated margin is also responsible for the irregularity of the marginal cutline. The dimple which appears upon the "anatomically, primary tuberculous intestinal ulcer" is first minute. It may be punctiform or longitudinal. Later it becomes gently crater-like, and still later it becomes rather flat bottomed with many small tubercules imbedded in the floor which is often thickened and upon which the tubercles are frequently visible, giving rise to a granular appearance. The floor may be of light greyish color; it may be interspersed by many ivory-colored, circumscribed, small tubercles; it may be covered by organized exudate-diphtheritic ulcer, or it may possess a blackish discoloration of either small or considerable extent. The discolored area may be dry, crusty, or soft and fragile. It is this type of ulcer that perforates. The greyish ulcer has the tendency to heal, and although it may often happen that the ulcer is deep, the thickness and firmness of its floor may be considerable.

I am not at this time going into details of the healing process of the intestinal ulcer. It has frequently been shown that these ulcers have a tendency to heal, and it may be assumed that certain far advanced tuberculous patients with no activity possess healed intestinal tuberculous lesions. We have found such upon postmortem examination.

The mucosa surrounding the ulcer may or may not be injected and may or may not show evidence of inflammation. When inflamed, it may be bright red or cyanotic. It may be edematous and of velvety thickness. It may show points of bleeding. The intestinal wall may be firm, elastic, or soft and collapsed. The serous surface may be of normal color or pale grey, grey, slaty grey, or almost black. It may be mottled by the traversing vessels in which stasis exists. The organ may be glistening but usually is lusterless. The location of the ulcer, if it be of some size, is recognizable upon external inspection of the intestine. There may be gentle bulging or thickening or retraction. There is usually localized peritonitis over it. The serous surface has a sandpapered appearance. The degree of stasis around the ulcer varies.

The recognition of small lesions is usually impossible upon inspection of the wall of the intestine, unless this wall is very thin and distended. We were never able to recognize small lesions from the outside. It is important to bear this in mind. Surgical removal of ulcerated lesions may promise a certain amount of benefit to the patient, especially when threatened with a stenosis, but the removal of such visible ulcers does not insure the removal of other ulcers near by, too small to be seen. We have seen at necropsy ulcers in the cecum and small intestine which were visible upon external inspection of the intestines. They were of the kind that even the best surgical judgment and the most conservative opinion would favorably consider operative interference with the hope of their complete eradication. Upon inspecting the open intestine, however, it was found that numerous small lesions existed as far up as the jejunum.

Microscopic examination of the early lesion shows the loss, or partial loss of epithelial cells of the mucosa. There may be débris still covering the subepithelial tissue. The lymph follicles show evidence of infection; the tubercles are found in the submucosa and chiefly composed of epithelioid cells. Giant cells are occasionally found in the more advanced lesions, but most frequently are not present during the early stage. In the more advanced tuberculous ulcer, necrosis, sometimes caseation, aggregation of round-celled infiltration, or typical tubercles might be found. The round-celled infiltration is usually in the submucosa, while the tubercles may be either in the submucosa, mucosa, or muscular layers. Round-celled infiltration is often marked beneath the epithelium. It is also found in the serosa, while tubercles might be found sometimes in the subserosa. When stained for tubercle bacilli, the organisms will be found not merely in the typical tubercle but also in the cluster of aggregated round cells. These clusters of round cells are frequently placed alongside a focus of early necrosis. The blood vessels are frequently engorged and dilated. Round-celled infiltration may or may not be generalized. The loss of tissue in the mucosa, submucosa, and muscularis depends upon the depth of the ulcer. Shallow ulcers will show no loss of the muscle tissue, although sometimes the intestinal wall is atrophied to the extent that even shallow ulcers will pass the muscle layers and reach the subserosa. At other times, the floor of the ulcer will be so studded by individual tubercles that it will give rise to considerable thickening of that floor. In the healing ulcer, fibrosis is a prominent feature.

Polypoid prolongation of the mucosa of the cecum and also in the colon is seen fairly often. We have seen it in 8 per cent of our cases upon necropsy. Sometimes only a few pale looking prolongations may be seen. At other times, however, the prolongations become diffused, especially in the cecum where their dense arrangement and considerable length cause almost complete obliteration of the cecal lumen in some cases. The mucosa has a shaggy appearance. The intestinal wall is thickened, firm, and rigid. A type of hyperplasia is seen chiefly in the colon, but also in the cecum. In this, the mucosa is of more or less velvety type. The organ is thickened throughout. The mucous membrane is granular and dense, yet soft and recoiling. The intestinal wall is comparatively inelastic. It is this kind of colon which is often markedly clean and free from fecal material, although the rectal ampulla as well as the small intestine is filled with stool, which at times is quite bloody. Because of the lessened absorbing surface and the probable hypermotility, the stool is not properly formed. This type of intestine will show microscopically many tubercles in the various structures. Ulcerative colitis, as a result of tuberculous infection, is also found, and tuberculous ulceration of the rectum is not unusual. A very frequent lesion is the tuberculous fistula in ano.

Colitis or ulcerative colitis, due to other causes than tuberculous infection may be present in the colon of the patient having pulmonary tuberculosis.

Perforation is a comparatively rare occurrence, considering the frequency with which ulceration of the intestines takes place. We had only three cases.

I have briefly given the pathologic picture of intestinal tuberculosis, with the intent to discuss a few points that may be of interest to the clinical pathologist who is called upon at times to assist in confirming the diagnosis of intestinal tuberculosis. The examination of a stool is usually requested in order to try to detect tubercle bacilli in it. Should such bacillus be found in a stool, its detection does not necessarily mean the existence of intestinal tuberculosis. It is a fact that the swallowing of the sputum containing tubercle bacilli is responsible much oftener than intestinal tuberculosis for the finding of the organisms in the stool. My purpose in describing the lesion both grossly and microscopically was to show that the débris which originates in a tuberculous ulcer of the intestine is of comparatively small amount, and this detritus contains either a very few tubercle bacilli or none at all. The examination of stools for tubercle bacilli, therefore, is not of great importance when the patient's sputum contains tubercle bacilli. The stool examination may offer some information, and it is of advantage to the clinical pathologist to be acquainted with the types of stool passed by the tuberculous patient.

Nothnagel[3] called attention to the fact that tuberculous lesions in the small intestine may be responsible for constipation, and lesions in the colon for the diarrhea sometimes complained of by the patient. This is also the opinion of Archibald.[4] In my experience, which coincides with A. Schmidt[5] and Tendeloo,[1] diarrhea of the tuberculous patient may be due to intestinal

tuberculosis, the site of the lesion being either in the small or in the large intestine. Extensive inflammatory processes of the small intestine in addition to the existing ulceration will give rise to an increased secretion, thus—diarrhea. On the other hand, extensive changes upon the surface of the colon will also result in diarrhea-like stools (soft—not formed) because of the lessened absorptive ability of the colon. Again, constipation is a frequent associate of intestinal tuberculosis, and the lesion may be in the small intestine, or at times in the cecum or colon. When present in the colon the lesions are comparatively limited in their extent, although individual lesions may be of considerable size. Ulcerations, whether present in small or large intestines, where not accompanied by extensive inflammation will not be responsible for the production of excessive secretion in the small intestine or greatly lessened absorption in the large intestine. On the contrary, with a lessened output of secretion in the small intestine and with slow passage of stools over a spastic colon, absorption of water will be more extensive. The stools will be drier than usual; in fact, scybalous masses may be found in the cecal pouch. Such masses when examined for occult blood will generally give a negative reaction, nor does the appearance of such stools suggest the presence of blood. There is, however, one kind of stool which does suggest the presence of blood. It is the one which is of oil emulsion-like thickness and of similar viscosity and of slaty grey color. It has the appearance of a thick fluid into which black gun powder was stirred. The test for blood will be positive to a very marked degree. Such stools may be present also in nontuberculous, acute enteritis (specimen Green). The history of the patient and the fact that he has tuberculosis of a far advanced degree, with considerable activity, suggests the possible diagnosis of intestinal tuberculosis. On the other hand, light brown, or sometimes alcoholic, smooth, pasty, viscous stool, which, although light in color, in fact sometimes of golden yellow color, often contains blood that is detectable by the usual methods, is considerable evidence in favor of the possible presence of intestinal tuberculous lesions. The cow dung like, soft, mushy, greenish dark stool which contains considerable admixture of gas is the product of fermentation rather than a sequel of intestinal tuberculosis. Fermentation is the result of either lessened hydrochloric acid in the gastric juice or lessened enterokinase in the enteric juice. The zymogen is not activated and tryptic digestion is lessened with resulting fermentation. Coprostasis, associated with fermentation, will give rise to the complaint of alternating diarrhea and constipation.

Hypochlorhydria is a fairly frequent condition, but is not evidence of intestinal tuberculosis. Blood counts, complement-fixation tests, or other blood tests are of but little assistance in diagnosing tuberculous ulceration of the alimentary tract. In tuberculous anal fistula it is possible to make a diagnosis. Scrapings of the fistulous tract, when stained with carbolfuchsin, will show the presence of tubercle bacilli in about 35 per cent of all cases.

SUMMARY

1. Intestinal tuberculosis is a frequent occurrence. It is usually secondary to a far advanced, active tuberculous infection of the pulmonary tissues.

2. Ulceration appears only after certain changes in the cells, which, as a result of part or complete devitalization, allow successful deposit and multiplication of the tubercle bacillus.

3. Early lesions may not be responsible for clinical manifestations, as complained of by the patient. In fact, such complaints may not be associated with intestinal tuberculosis; or there may be no complaints whatsoever at the time the lesions make their appearance.

4. The early dyspeptic manifestations do not represent the symptoms of intestinal tuberculosis. It is during the stage of spasticity and peritoneal irritation that symptoms arise resulting from tuberculous intestinal infection.

5. The ulcers are first small and circular; later they are large and of irregular outline. They may follow the course of the mucous fold and form the encircling ulcer. They may traverse a number of folds and then represent the longitudinal ulcer.

6. Perforation is not frequent as there is a tendency towards thickening of the floor of the ulcer.

7. In the cecum and colon there may be either individual lesions or extensive diffuse tuberculosis, with thickening of the entire wall of these organs.

8. Bleeding occurs at some time during the course of ulceration.

9. Diarrhea, due to tuberculosis, is not always a result of lesions in the colon, neither is constipation always a result of ulceration in the small intestine.

10. The finding of tubercle bacilli in the stool rarely, if ever, confirms the existence of intestinal tuberculosis.

11. The gross character of the stool should be studied when intestinal tuberculosis is suspected. The nonfermentative pasty, alcoholic, viscous stool, especially the one containing occult blood, should be looked upon with suspicion, as it may point towards tuberculous intestinal infection. The findings must agree with the clinical findings and the patient's history of active tuberculosis.

12. There is no known clinical laboratory test by means of which a diagnosis of intestinal tuberculosis can be made.

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BRAIN STRUCTURE AND BLOOD CHANGES AFTER TREATMENT IN GENERAL PARALYSIS*

By A. M. Saunders, M.D., Dunning, Ill.

THE treatment of general paralysis by inoculation with Plasmodium malariae, brought before the medical world through von Jauregg, has aroused wide interest and is one of the most outstanding achievements in the treatment of disease in recent years.

Since the malaria treatment has been instituted in the Illinois state hospitals, a marked change in the appearance of the wards for paretics has taken place. Formerly a case of general paralysis meant a bedridden, foul-smelling, untidy patient, requiring complete care for from three to five years or until he died. With the exception of a few, all of those treated patients are able to be out of bed and can take care of their personal needs. Many of them assist with the work on the ward and some of them go out during the day to work in the industrial departments. Quite a large number can go home and return to their former occupations.

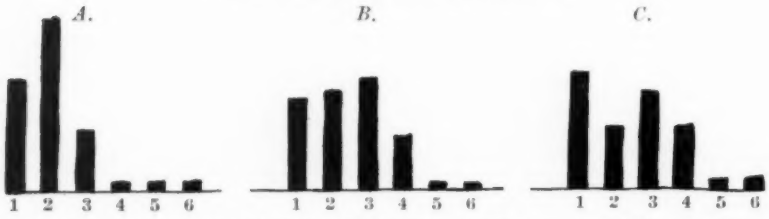
It has been said that with the malarial treatment the paretics make a "good social adjustment," but no changes in the brain pathology, in the blood, and in the spinal fluid have been found. During the last year, this has been found to be incorrect. Straussler and Koskinas, in their study of thirty-eight brains of patients with general paralysis who had been treated with malaria, have found only a very mild degree of inflammation. It is their belief that the characteristic diffuse inflammation of a typical paretic brain changes to a more localized, gummatous type, resembling cerebrospinal lues. Gerstmann finds that mental improvement in those cases corresponds with changes in the brain. Nicole and Steel have often obtained negative serologic results on blood and spinal fluids in patients treated with Plasmodium malariae, but were unable to prove any relationship between the serologic findings and the mental and physical improvement. Some patients with good mental and physical results still had a positive Wassermann and Lange test, while others with only fair mental and physical changes had a negative Wassermann on both blood and spinal fluid. This observation has been substantiated at our Psychopathic Institute. Dr. G. B. Hassin, at our Institute, has found a regression of inflammation after treatment with the malarial organism and tryparsamide. In no case was a regeneration of the parenchyma found, but the changes in the meninges, and especially in the perivascular cell infiltration, have been very striking. The marked decrease, or even absence, of plasma cells, which in untreated cases crowd the lumen of the cerebral capillaries, has been most interesting. The plasma cells were replaced by lymphocytes of the small cell type.

*Read before the Sixth Annual Convention of the American Society of Clinical Pathologists, Washington, D. C., May 13, 14, and 16, 1927.

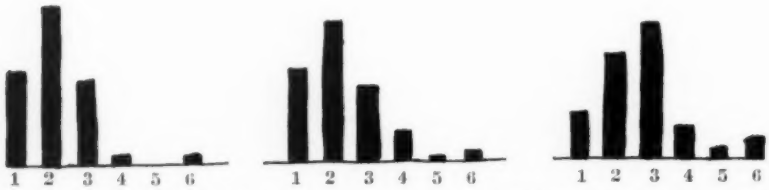
From the Illinois State Psychopathic Institute.

ARNETH COUNT

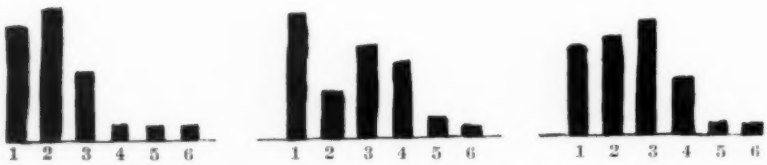
A. Before antiparetic treatment; *B.* three months after malaria treatment; *C.* one year after malaria treatment.



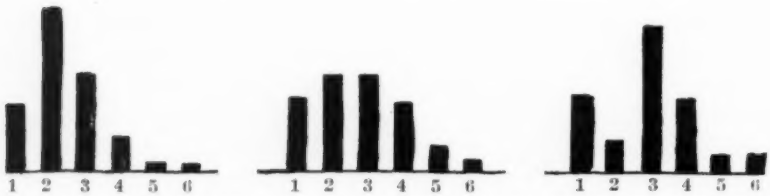
Case 1.—Mental and physical improvement good; no serologic change.



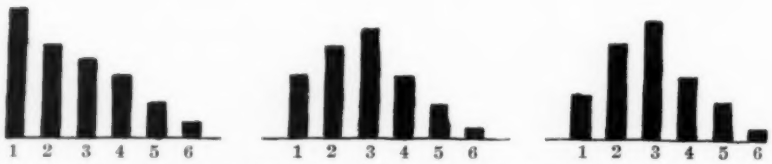
Case 2.—Mental and physical improvement good; moderate serologic improvement.



Mental improvement poor, physical fair; no serologic change.



Physical and mental improvement fair; serologic findings negative.



This patient has been physically, mentally, and serologically normal for eighteen months after treatment.

Fig. 1.—Column 1 indicates relative number of neutrophiles with one nucleus.
Column 2 neutrophiles with two nuclei.
Column 3 neutrophiles with three nuclei.
Column 4 neutrophiles with four nuclei.
Column 5 neutrophiles with five nuclei.
Column 6 eosinophiles.

Following the observation of changes in the type of cells in the brain we reasoned that it might be interesting to see if there was a definite change in cells in the blood also and we have made regular examinations of blood smears from patients with general paralysis, before any treatment was given, three months after malaria treatment, and again one year after the treatment. We have found the relative number of small lymphocytes very variable and probably influenced by the malarial organism, but we failed to find any relationship in the number of small lymphocytes to the physical and mental reaction to treatment. There also was no relationship demonstrable in the number of eosinophiles to the condition of the patient. The Arneth count, however, yielded very definite and constant results in over one hundred and twenty cases, showing a shifting to the right after malarial treatment, usually reaching its maximum within twelve months. In many cases the marked shift to the left found in untreated cases of general paralysis had been corrected to such an extent as to give a nearly normal Arneth count. The following charts (Fig. 1) will illustrate only a few but quite characteristic and constant changes in the Arneth count before malaria treatment and at definite intervals afterward, covering a period of one year. This shift to the right in the Arneth index was found in over 80 per cent of a total number of one hundred and twenty cases; reaching its maximum after from six to twelve months after the intravenous introduction of plasmodium malariae.

DISCUSSION

Dr. G. B. Kramer.—I merely want to ask Dr. Saunders how many chills a patient must have before antimalarial treatment is instituted.

Dr. Saunders (closing).—We usually wait until the patient has had twelve definite chills.

A STUDY OF THE ATMOSPHERIC POLLEN AND BOTANIC FLORA OF THE EAST SHORE OF SAN FRANCISCO BAY*

By Albert H. Rowe, M.S., M.D., Oakland, Calif.

THIS paper reports observations and deductions made throughout the last twelve months on the atmospheric pollen and on the botanic flora of the cosmopolitan area on the eastern shore of San Francisco Bay. A study of the flora of San Francisco itself shows that it differs in only a few respects from that of the East Bay district which we shall consider.

The East Bay district is in central California and extends between 37° 6′ and 38° latitude, from Haywards on the south to Richmond, which is thirty miles to the north. This area is bounded on the west by San Francisco Bay and is from four to seven miles in width, with the crest of the coast range hills rising to a height of from 1000 to 2000 feet at the eastern border. Along the bay shore, salt marshes are found in Richmond, Alameda, San Leandro, and Haywards. The inhabited area is either flat or moderately rolling, while the hills to the east are cut by wooded canyons that have much grass and Artemisia Californica and Vulgaris over them, and have in places pines and eucalyptus which have been planted. In one area, there are native redwood trees. The temperature varies between 40° F. and 75° F. throughout the year. The prevailing winds are from the west and south and occasionally from the north. The rainfall averages about 25 inches, a few rains occurring in the fall and spring and a good many during the winter period. Winters are mild, and only rarely does snow fall on the hills. The climate of this district, especially of Alameda, Oakland, and Berkeley, is tempered in the summer by sea breezes and high fogs which roll in over San Francisco Bay and through the Golden Gate across the bay, which is a body of water varying from 3 to 7 miles in width. This mild, dry climate, with the prevailing winds off the bay, explains in part the presence of small amounts of pollen in the air, especially in the summer and fall.

The East Bay region is in the center of the Franciscan botanic area, described by Jepson[1] as extending from Monterey on the south to Mendocino County on the north. This area contains many endemic plants, as well as many alien exotic species, especially grasses, which have been brought into this region chiefly from the Mediterranean countries.

PLAN OF STUDY

A combined study of the atmospheric pollen and of the botanic flora of this large cosmopolitan area was suggested by the article of Koessler and Durham[2] on their survey of Chicago. During the summer and fall of 1926, a botanist, Miss Weisendanger, gave her entire attention to the problems of this

*Read before the American Association for the Study of Allergy, Washington, D. C., May, 1927.

report, and plate counts of atmospheric pollen have been continued by my assistant, Mrs. D. Fletcher, during the remainder of the twelve-month period. Our data on the pollen content of the air at four different stations in this East Bay district constitute the first data of this type gathered over a period of an entire year.

This district has been divided for convenience of study and tabulation into seven areas, by boundaries of the separate communities of this area, whose total population is about five hundred thousand people. These districts are from north to south, Richmond, Berkeley, Oakland, Piedmont, Alameda, East Oakland, and San Leandro and Haywards, as indicated roughly in Chart 1.

Chart 1.

MORPHOLOGY OF POLLEN

In order to identify the pollens on the exposed plates, it was necessary at the outset of our work to become familiar with the size, shape, and external features of the pollen grains of all the common hay fever-producing plants of this East Bay district. The articles of Wodehouse[3] and Pope[4] are the only recent contributions on the morphology of pollen. Both articles include drawings of various pollens, but no definite drawings of California pollens are available. My bacteriologist, Miss Lundahl, has made careful drawings of the various types of dry pollens. These are reproduced in Chart 2 and are drawn according to scale. Pollen grains adhering to slides covered with white

vaseline maintain their dry shrunken shapes, and our studies for identification purposes have therefore been made on the dry pollens.

Miss Pope has classified the pollen grains of a large number of families of plants according to structural features. On the basis of her classification and on our own observations, I have classified the common hay fever-producing pollens of this East Bay District in Table I.

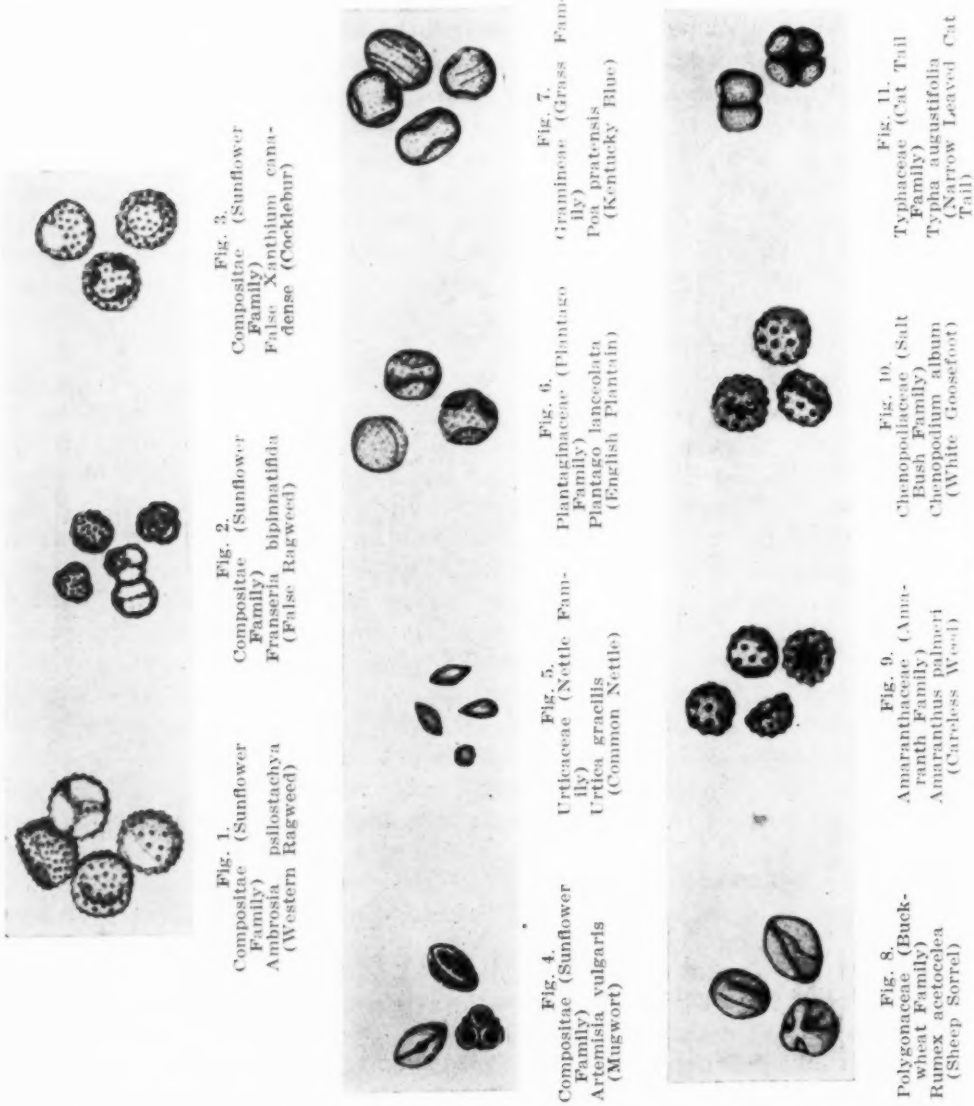


Fig. 1. Compositae (Sunflower Family) Ambrosia psilostachya (Western Ragweed)

Fig. 2. Compositae (Sunflower Family) Franseria bipinnatifida (False Ragweed)

Fig. 3. Compositae (Sunflower Family) False Xanthium canadense (Cocklebur)

Fig. 4. Compositae (Sunflower Family) Artemisia vulgaris (Mugwort)

Fig. 5. Urticaceae (Nettle Family) Urtica gracilis (Common Nettle)

Fig. 6. Plantaginaceae (Plantago Family) Plantago lanceolata (English Plantain)

Fig. 7. Gramineae (Grass Family) Poa pratensis (Kentucky Blue)

Fig. 8. Polygonaceae (Buckwheat Family) Rumex acetocelea (Sheep Sorrel)

Fig. 9. Amaranthaceae (Amaranth Family) Amaranthus palmeri (Careless Weed)

Fig. 10. Chenopodiaceae (Salt Bush Family) Chenopodium album (White Goosefoot)

Fig. 11. Typhaceae (Cat Tail Family) Typha augustifolia (Narrow Leaved Cat Tail)

Chart 2.

The drawings and this table of the characteristics of the various pollens indicate that it would be difficult to distinguish with certainty the identity of the spiculated pollens belonging to the Compositae family, though we feel that we can usually pick out ambrosia pollen quite accurately. Artemisia pollen would be difficult to separate from the ellipsoidal smooth tree pollens

were it not for the different seasons of pollination. Plantago and Rumex pollens are probably often confused, though the bands on the Rumex pollen and

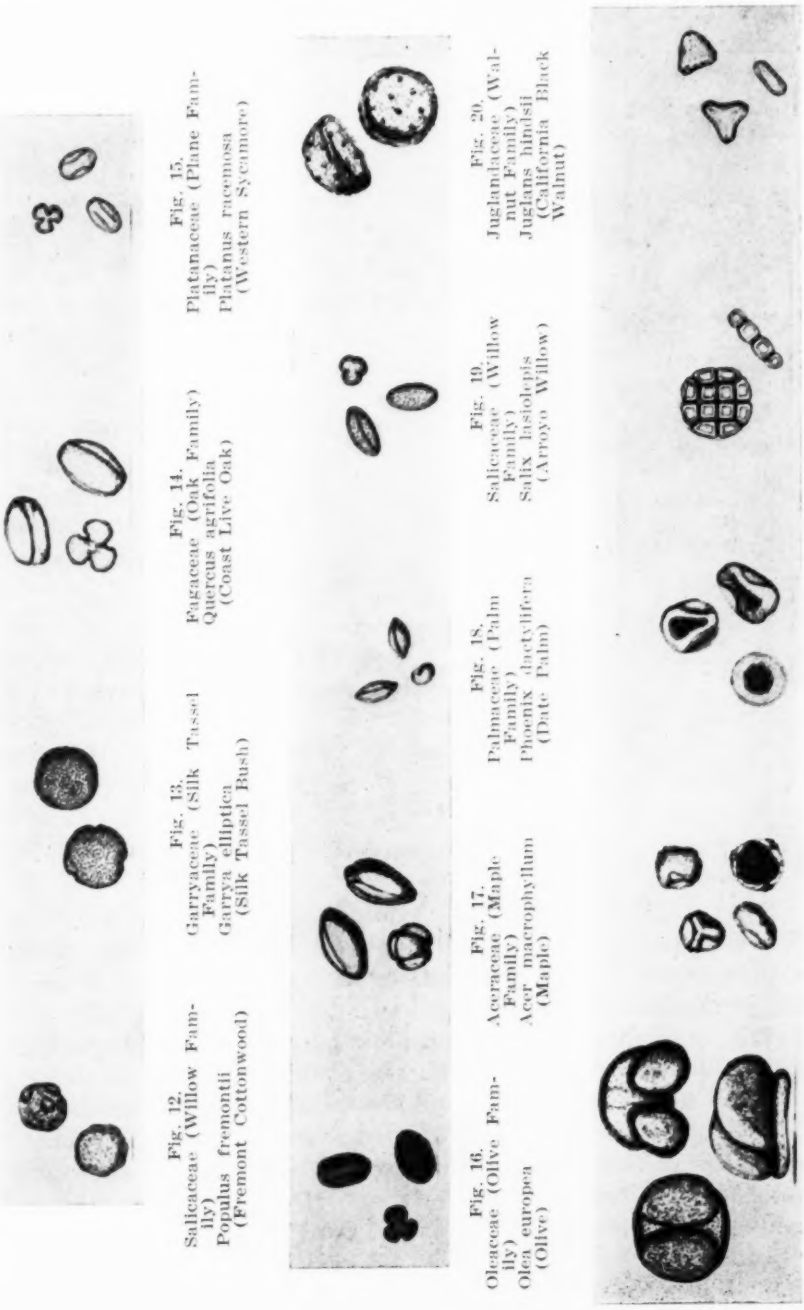


Fig. 12. Salicaceae (Willow Family) Populus fremontii (Fremont Cottonwood)

Fig. 13. Garryaceae (Silk Tassel Family) Garrya elliptica (Silk Tassel Bush)

Fig. 14. Fagaceae (Oak Family) Quercus agrifolia (Coast Live Oak)

Fig. 15. Platanaceae (Plane Family) Platanus racemosa (Western Sycamore)

Fig. 16. Oleaceae (Olive Family) Olea europea (Olive)

Fig. 17. Aceraceae (Maple Family) Acer macrophyllum (Maple)

Fig. 18. Palmaceae (Palm Family) Phoenix dactylifera (Date Palm)

Fig. 19. Salicaceae (Willow Family) Salix lasiolepis (Arroyo Willow)

Fig. 20. Juglandaceae (Walnut Family) Juglans hindsii (California Black Walnut)

Fig. 21. Pinaceae (Pine Family) Pinus radiata (Monterey Pine)

Fig. 22. Betulaceae (Birch Family) Betula papyrifera (Paper Birch)

Fig. 23. Cupressaceae (Cypress Family) Cupressus macrocarpa (Monterey Cypress)

Fig. 24. Leguminosae (Pea Family) Acacia

Fig. 25. Eucalyptus.

Chart 2—Continued.

its ellipsoidal shape are quite different from the features of Plantago pollen. Amaranth and Chenopod pollens are so similar that they cannot be distin-

TABLE I

TABULATION OF CHARACTERISTICS OF HAY FEVER-PRODUCING DRY POLLEN GRAINS OF THE EAST BAY REGION OF CALIFORNIA

	SHAPE	TYPE	AVERAGE SIZE (μ)	FIGURE*
I. Shrubs, grasses and weeds				
A. Echinate (Probably all Compositae except Artemisia)	1. Spherical	Ambrosia	38	1
		Franseria	25	2
		Xanthium	34	3
		Iva	25	–
		Helianthus	38	–
	2. Ellipsoidal	Hemizonia	38 × 29	–
B. Smooth	1. Ellipsoidal (Polyhedric)	Artemisia	38 × 25	4
		Urticaceae	25 × 12	5
		Ricinus	46 × 33	–
	2. Spherical	Plantago	34	6
	3. Polyhedric	Gramineae	32 × 40	7
C. Punctate, with Furrows		Brassica	46 × 25	–
D. With Bands	1. Ellipsoidal	Rumex	46 × 34	8
E. With Distinct Pores	1. Spherical	Amaranthus	29	9
F. With Less Distinct Pores	1. Spherical	Atriplex	29	10
		Chenopodium	29	–
		Salicornia	29	–
G. Four Grains Held Together		Typhaceae	10	11
II. Trees				
A. Punctate	1. Spherical	Populus	29	12
	2. Ellipsoidal	Garryaceae	38	13
B. Three-Lobed	1. Smooth			
	a. Blunt ends	Fagaceae	42 × 29	14
		Plantanaceae	25 × 17	15
		Oleaceae	33 × 21	16
	(Markedly reticulate)			
	b. Less blunt	Aceraceae	46 × 25	17
	c. Pointed ends (One Groove)	Palmaceae	25 × 13	18
	2. Punctate	Salix	29 × 18	19
C. Hemispherical		Juglandaceae	50	20
D. Two-Winged	1. Reticulate	Pinaceae	63	21
E. Polyhedric	1. Smooth	Betulaceae	29	22
		Cupressaceae	32	23
F. Triangular	1. Smooth	Eucalyptus	25	24
G. Gridiron	1. Spherical	Acacia	42	25

*This refers to figure numbers on Chart 2.

guished accurately. All pollens of the Chenopodaceae family are spherical, about equal in size, and have fairly definite depressions on their external surfaces. We have found Gramineae pollens of the same characteristic shape, and feel that we can identify them quite accurately on the pollen plates. Our drawing is of the dry pollen and, therefore, does not show the single germinal aperture which Wodehouse points out is the distinguishing feature of the Gramineae pollens in the wet swollen condition. Poa annua, Festuca myuros, and Cynodon dactylon pollens are on the average smaller than the pollen of many other species of Gramineae. However, such difference in size cannot form the basis of really accurate differentiation. No difficulty is experienced in recognizing most of the tree pollens, except those already mentioned.

ATMOSPHERIC POLLEN

Pollen counts have been made twice every week throughout the year in four of the districts shown in Chart 1; namely, Richmond, Oakland, Piedmont, and East Oakland, and in five other locations in this East Bay region twice

every week from May to October of 1926. Microscopic slides covered with a thin coating of white vaseline were exposed in an upright position toward the prevailing wind for twenty-four hours. One square inch of each slide was carefully examined microscopically, and the various pollen grains on these areas were counted and identified. The amount of pollen on one square inch of plate surface is recorded rather than that on 0.55 square inch, as reported by Koessler and Durham, because of the small amount of pollen present in the air of this East Bay region as compared with that in the air of many areas in the middle western and eastern states.

The average counts of all stations for each day that slides were exposed throughout the year are recorded in Table II. The number of slides counted on each day is indicated, together with comment on the wind and weather. The usual occurrence of high sea fogs and the absence of many excessively hot days in this district is shown in the table. Rain occurred for a few hours on many days during which slides were exposed and used for our data. When rain continued all day, no exposed slides were counted, which accounts for a few irregularities in the routine of pollen counting in the winter.

On the basis of the studies of the morphology of the hay fever pollens of this district, the pollens have been classified under the groups indicated in Table II. The reasons for such grouping of pollen grains are given in the discussion on morphology of pollen in this district.

The data in Table II consisting of 8 or 9 observations for each month have been condensed into average weekly values and are presented in a graphic manner in Chart 3. A study of this chart shows that the height of the grass season occurs in the last two weeks of May and the first week of June, due to the profuse pollination of Lolium perenne, as shown by field observations. The chart shows that grass pollen is in the air, however, throughout the year, the smallest amount being present during August, September, and October. With the onset of rain, in September, a slight increase in grass pollen occurred. On sunny, mild days throughout November, December, January, February, and March, grass pollen was found in increasing amounts in the air, and persisted through the spring. The types of grass pollen making up the average curve will be discussed under the section on the flora.

The occurrence of tree pollens in the air is tabulated separately in Table II and shown as a group in Chart 3. Such pollen begins to appear in the last week of January and continues on through February, March, April, May, and June. Pine pollen, as shown in Table II, has been found in small amounts throughout the year, due to the many species of pine found in the parks and gardens and on the hills of this district. Pine pollen apparently does not cause hay fever or asthma and consequently has not been included in Chart 3. An independent curve for willow pollen is shown during March. This pollen probably causes little if any allergy. Rumex and Plantago pollens were in the air during February, March, April, May, and June, especially in April and May, corresponding with field observations. Chenopod and Amaranth pollens were present in very small amounts during May, June, July, August, September, and October, showing that in this region these species do not produce much pollen, in spite of their frequent occurrence in some districts. Artemisia

TABLE II

AVERAGE POLLEN PLATE COUNTS OF ALL STATIONS

	GRAMINEAE	RUMEX AND PLANTAGO	ARTEMISIA	OTHER COMPOSITAE	ZEA MAYS	AMARANTH AND CHENOPOD	SALIX (WILLOW)	ACACIA	PINACEAE	TYPHA	EUCALYPTUS	FAGACEAE	CRUCIFERAE	JUGLANDACEAE	PALMACEAE	BIRCH	MAGNOLIA	MISCELLANEOUS	PLATES EXPOSED	TOTAL AVERAGE PLATE COUNT	WIND	WEATHER CONDITIONS
1927																						
Jan.	4	13						1											8	14	W	LF*
"	10	24																	1	24	SW	
"	12	3		7	1										2				8	13	W	Cold
"	17	1																	1	1		Cl.*
"	21	3																	6	3	S	Cold
"	24	2			1														5	3	S	Cold
"	28	40	1	2				2							2				6	47		Rain
"	31	6																	3	6		Rain
Feb.	4	7	1																6	8	S	
"	7	6	1												1				6	8		Clear
"	10	15	7					2							2				6	26	S	Rain
"	14	1	1					2							4				4	8	N	Rain
"	24	3	1					1											6	5		Rain
"	26	10					16		20						16				1	62	S	
Mar.	1	1							3										6	4		Cl.
"	3	1							2										1	3		Rain
"	7	15					6	1	55										5	77	S	Rain
"	10	6							1										6	7		Cold
"	15	5						1	8										5	14	N	
"	18	4	6				171	1	4				1						6	187	N	
"	22	1						1	1				1						6	4	N	
"	24	4						1	1				1						6	7	S	
"	28	14						1	2										6	17	N	
"	31							4	4										1	8	N	Rain
Apr.	5	19	1				2	1				3							6	26		Cold
"	7	39	8				8	4	3			8	1		1				6	33	N	Rain
"	15	1	6						1			5	1					2	5	16		HF*
"	18	3	4				2		1			6			2				5	18	S	
"	22	12	10				3		1			9							6	35	S	
"	25	10	17				3		2			6			4				6	42	N	
"	28	41	31				2		1			7							6	82	W	
May	2	25	16				1		2			4							6	48	W	
"	5	9	9						1			2							6	21	N	Rain
"	17	26	7				1		3									3	8	40	N	
1926																						
May	20	94	10			1		1	2									2	9	111	N	
"	24	54	3			1		1	1									1	8	61	N	Cold
"	27	78	13			1		1	4		1							2	9	100	N	
June	1	53	9					1	2									1	8	66	W	
"	3	52	7			1		1	3	1								1	9	66	S	Cold
"	7	16	3			1		1	2	1								1	9	25	S	Cl.
"	10	33	9			1		1	1									1	7	46		Cl.
"	14	24	3			1		1	2									1	9	32	N	
"	17	12	2		1	1		1	2									1	9	20	W	Cold
"	21	12	4			1		1	1				1					2	10	22	N	
"	24	16	5			1			1				1					1	8	25	S	Cl.
"	28	8	1						1									1	9	12	N	
July	1	6	1		8				1									2	9	18	N	
"	6	8		1					1										8	10	W	Cold
"	8	3							1				1						9	5	W	HF
"	12	3		1		1													6	5	W	

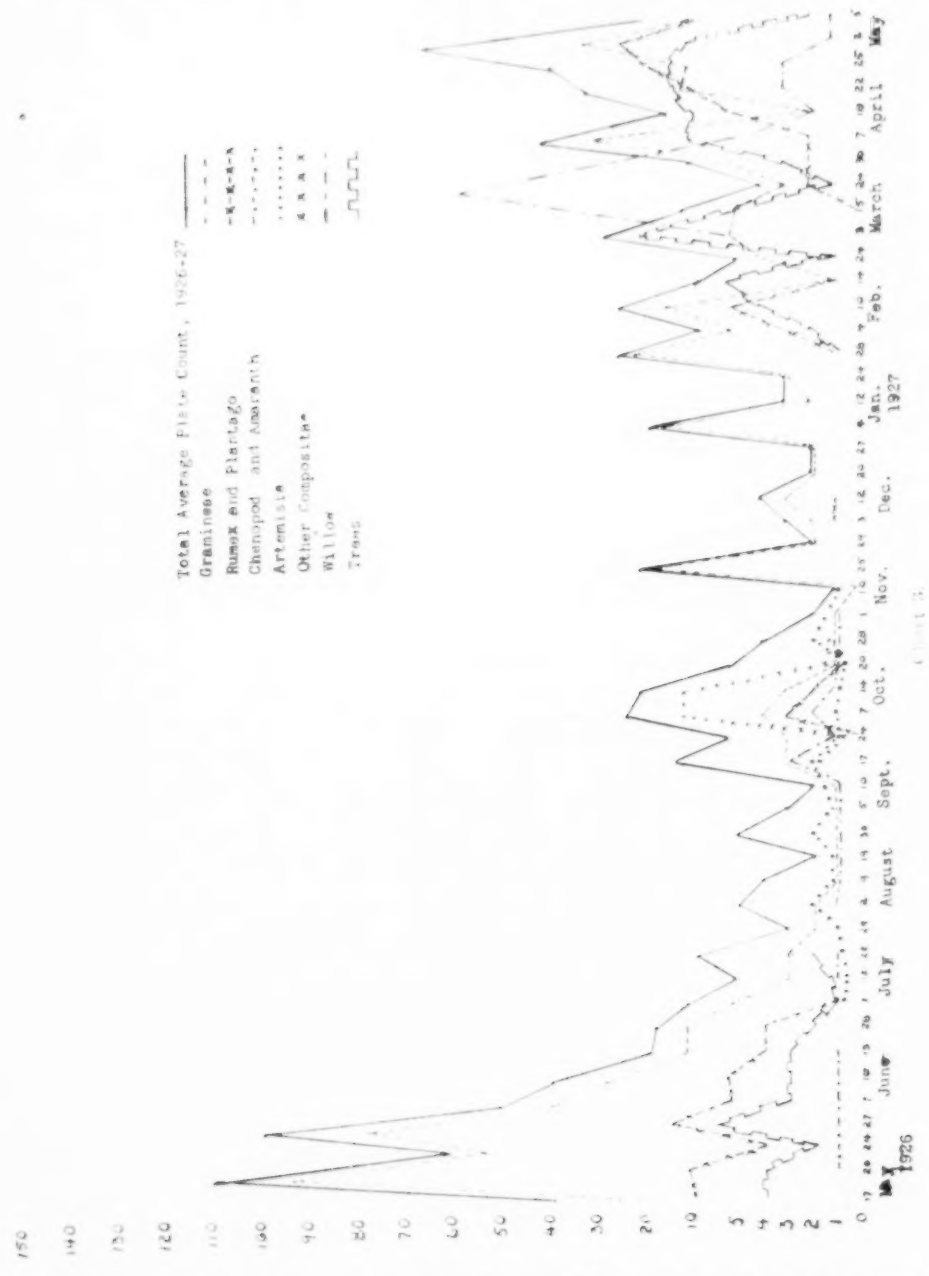
*LF—Low Fog; HF—High Fog; Cl.—Cloudy.

TABLE II—CONT'D

		GRAMINEAE	RUMEX AND PLANTAGO	ARTEMISIA	OTHER COMPOSITAE	ZEA MAYS	AMARANTH AND CHENOPOD	SALIX (WILLOW)	ACACIA	PINACEAE	TYPHA	EUCALYPTUS	FAGACEAE	CRUCIFERAE	JUGLANDACEAE	PALMACEAE	BIRCH	MAGNOLIA	MISCELLANEOUS	PLATES EXPOSED	TOTAL AVERAGE PLATE COUNT	WIND	WEATHER CONDITIONS
July	15	4					1			1				1					1	10	8	W	
"	19	4		5			1			1									1	10	12	W	
"	22	2			1	1	1													9	5	W	
"	26	2		1						1										9	4	W	
"	29	2		1																10	3	W	
Aug.	2	2		1			1													10	4	W	HF*
"	5	2		1			1													11	4	W	HF
"	9	3		3																9	6	W	HF
"	12	2		1			1													9	4	W	HF
"	17	1																		9	1	S	HF
"	19	1		2	1		1													8	5	W	HF
"	23																			9	0	W	HF
"	26	1		1			1			1										10	4	NW	Rain
"	30	2		3			2		1	1										8	9	W	
Sept.	2	1		1	1				1	1									1	10	6	W	HF
"	7	1		1			1													10	3	W	
"	10	1		1			1													7	3	W	HF
"	13	1					1													8	2	W	HF
"	17	5		2	4		2											1		2	14	N	
"	21	3		4	2		2											1	6	3	18	S	
"	23	2			1		2												1	4	6	S	
"	24	1			1		1												4	2	7	N	
"	25																			1	0	W	
"	27	9		1	1		1			1										3	13	W	
"	30	3		1	2		2											1		4	9	W	Rain
Oct.	3	2	3	2	12		3		1									1		7	24	S	
"	7	7	3	1	13		1		1	1										7	27	S	
"	10	5	3		23		1								7		1			7	40	SW	
"	14	1	1	1	1				1	1										7	6	S	
"	18	2	2	1	1															7	6		HF
"	21	1	1	1			1			1										8	5	SW	
"	25	1		2	1		1													7	5	SW	
"	27	3							1											1	4	N	
"	28	1		1	1		1													7	4	SW	
Nov.	1	1	1	1	1				1											7	5	NW	
"	4	2		1	1		1			1										8	6		LF*
"	8	1	1	1	1				1											8	5	N	
"	11	1		1	1		1			1										7	5		Rain
"	15	1	1	1			1													7	4	NE	
"	18			1																3	1		LF
"	22	42																		1	42	W	
"	25	1					1													3	2	S	Rain
"	29	2		1																3	3		Rain
Dec.	3	1	1	1	1															7	4	N	
"	6	2	1						1									1		6	5	W	
"	7	3	2																	2	5	N	
"	8	2	1	1					1									1		3	6		HF
"	12	2	1	1																7	4	N	
"	15	1	1							1										7	3	SW	
"	20	3								1										8	4	N	
"	27	2																		8	2	W	Rain

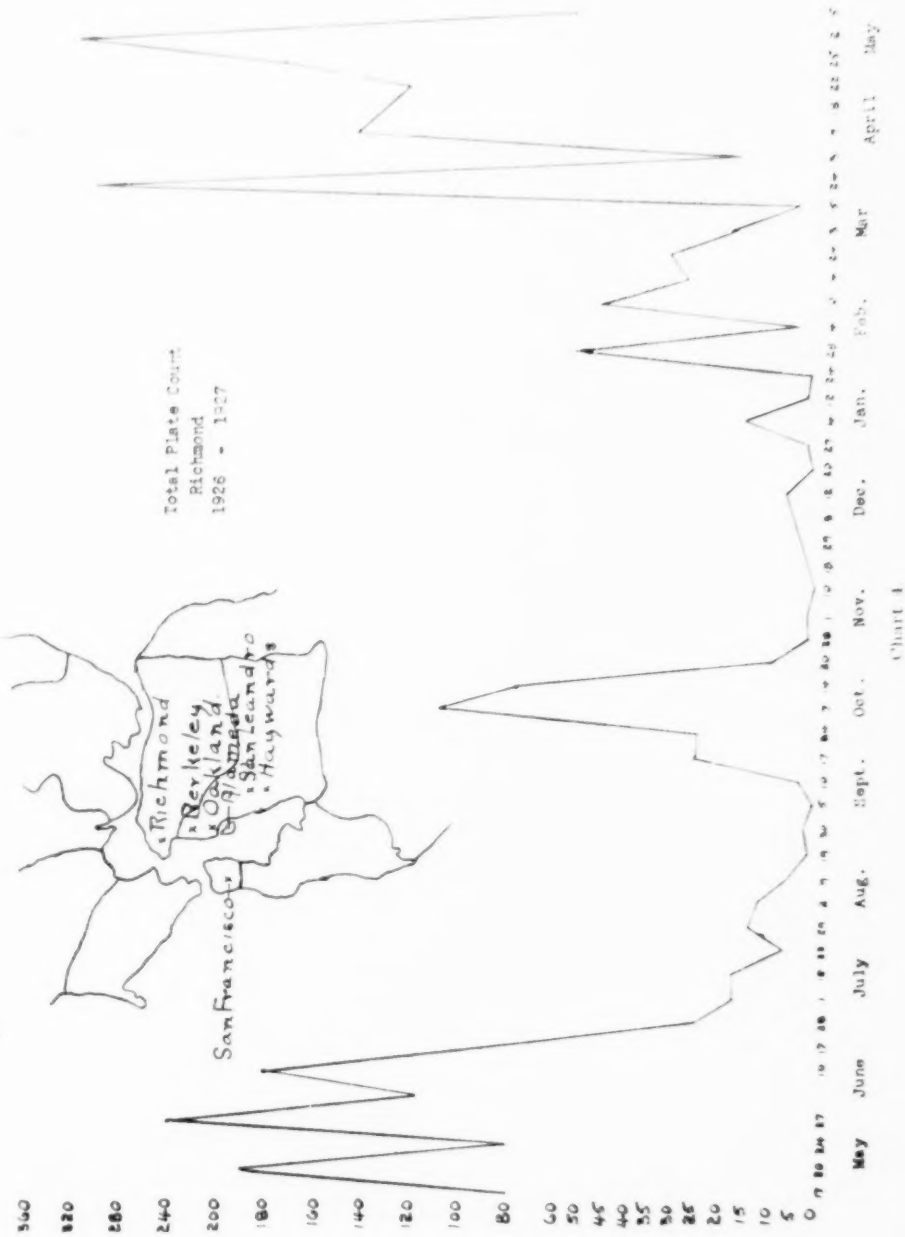
*LF—Low Fog; HF—High Fog; Cl.—Cloudy.

pollen likewise was not abundant, though present in the air during July, August, September, and October. Pollen of other Compositae, which was probably largely Ambrosia pollen, was found in the last week in September



and during October. Franseria pollen from field observations would constitute a considerable part of the Compositae pollen in those districts shown in Table V, to contain Franseria Bipinnatifida. The possibility must be kept in

mind that pollen of many species growing at a distance from this East Bay region may be brought into the air through the action of upper air currents. This possibility has not been studied, but seems unlikely because of the nar-



row district under consideration, extending from the bay line to the high eastern barrier of hills, the elevation of which is between 1000 and 2000 feet, and because of the prevailing westerly or southwesterly winds. Moreover,

with the occasional hard dry north winds, it was found that the character and amount of atmospheric pollen did not change in any definite manner.

Four separate charts of total average pollen counts for each week

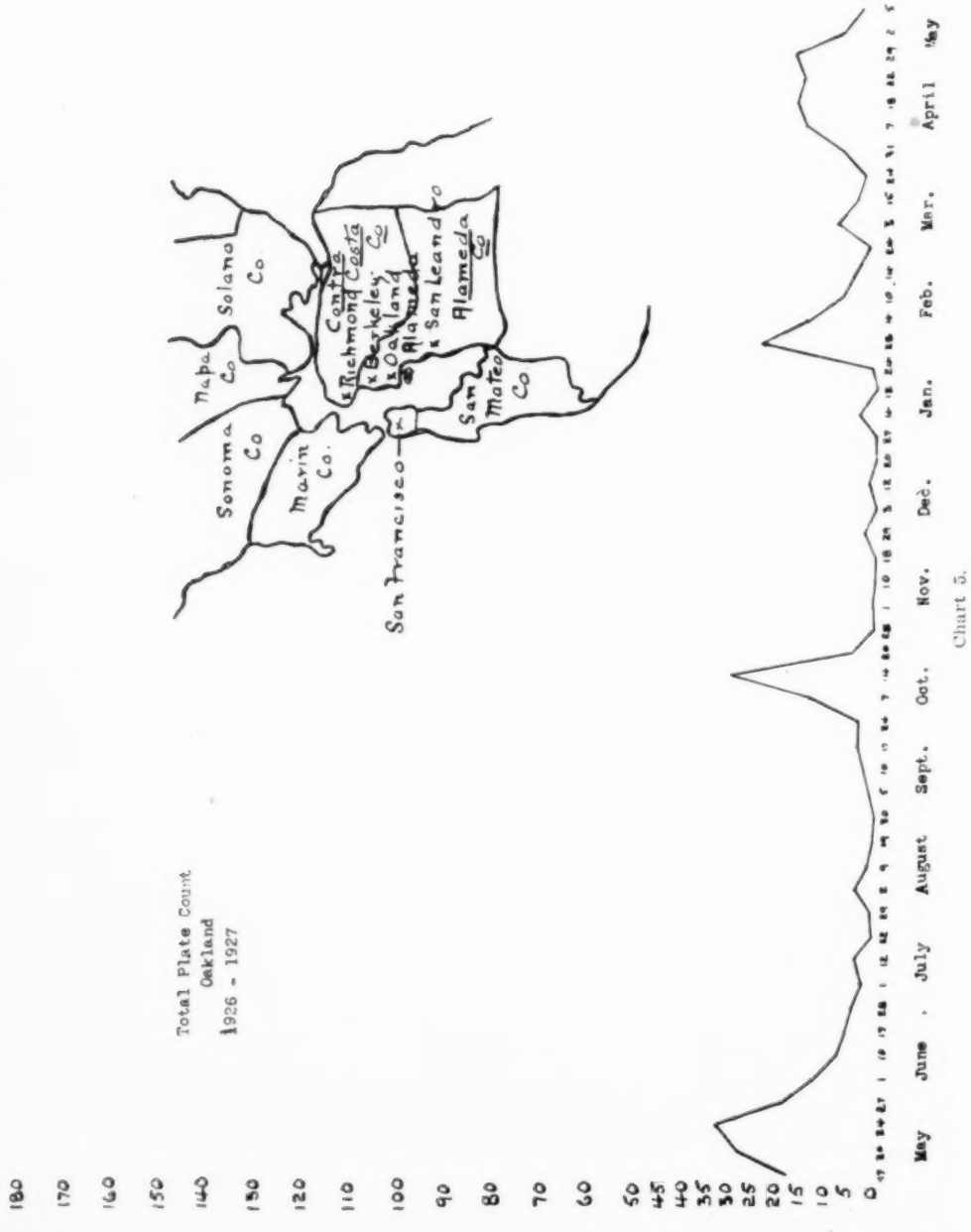


Chart 5.

throughout the year are presented in Charts 4, 5, 6, and 7, for Richmond, Oakland, Piedmont, and East Oakland respectively. The individual groups of pollens, as shown in Chart 3, were determined in the case of each district.

The mass of data obtained does not permit of tabulation in this article, and only charts of total pollen counts are presented. The location of each district with reference to the territory around San Francisco Bay is shown in

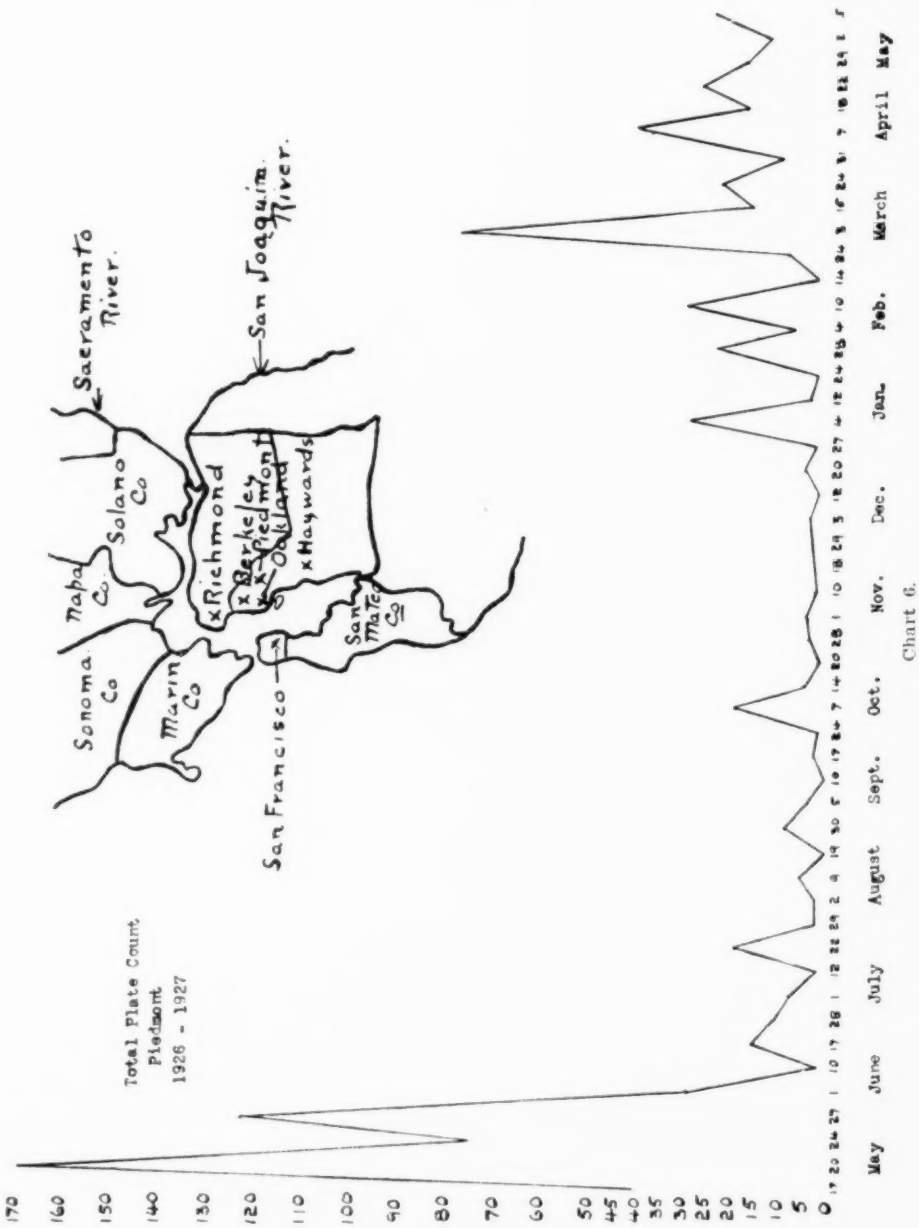
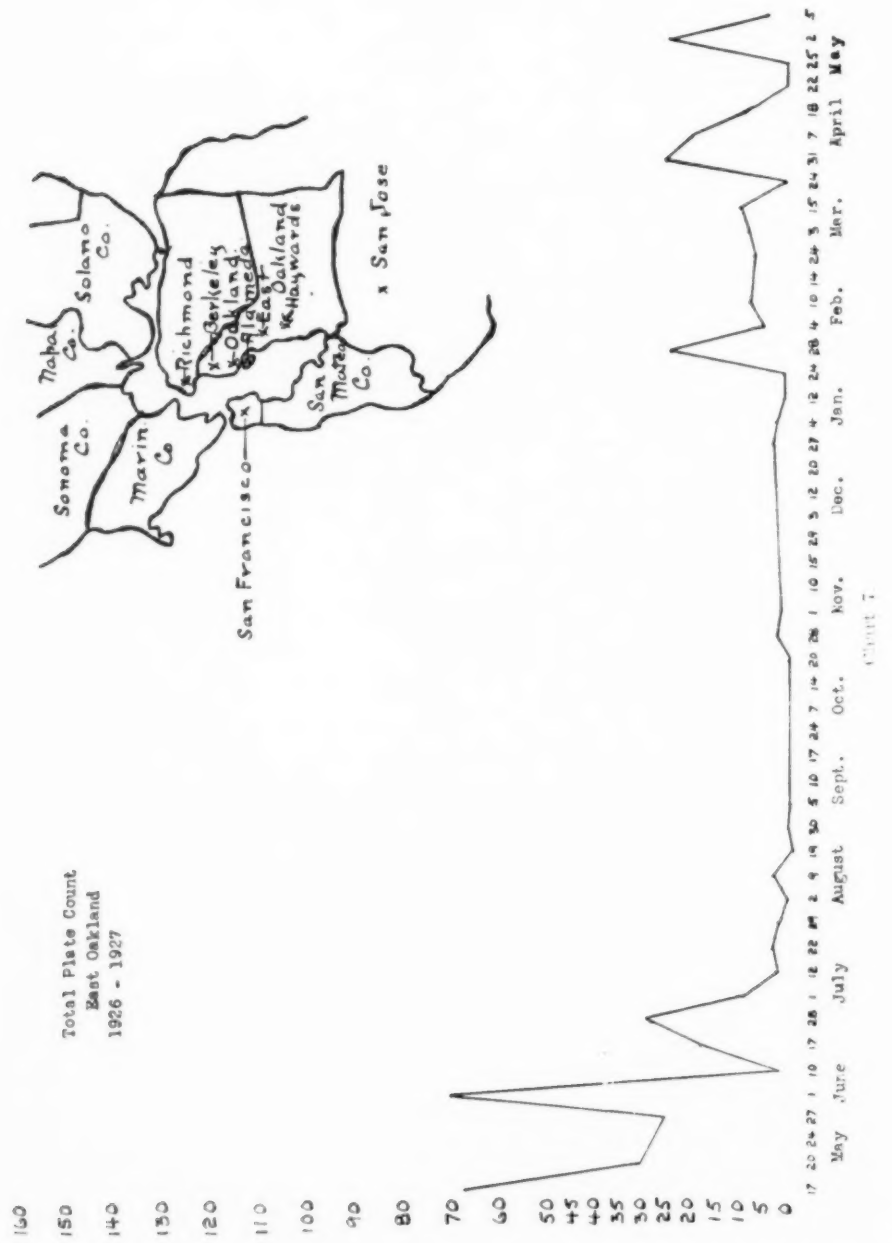

Sacramento River
San Joaquin River
Solano Co
Napa Co
Sonoma Co
Marin Co
San Francisco
San Mateo Co
Richmond
Berkeley
Piedmont
Oakland
Haywards
Total Plate Count, Piedmont 1926 - 1927
May June July August Sept. Oct. Nov. Dec. Jan. Feb. March April May
170 160 150 140 130 120 110 100 90 80 70 60 50 45 40 35 30 25 20 15 10 5 0

Chart 6.

the regional map on each chart. These charts show that Richmond, which has large tracts of vacant land and is subject to much wind, has the highest counts. The count of 308 in March was largely due to willow pollen and about 50 per

cent of the count of 322 in May was due to grass pollen and 50 per cent to Rumex and Plantago pollens. The peak of 115 in October was due to Compositae pollen, which was largely Ambrosia rather than Franseria or other



Compositae pollen, as determined by field studies. The chart of Oakland shows a comparatively small amount of atmospheric pollen throughout the year. The peaks correspond to those in Chart 3, and the constituents of the

total curve in this, as well as in the other charts, in a general way corresponded to the average values shown in Chart 3. The chart of Piedmont shows higher counts than those obtained in Oakland. The general conclusions in regard to Chart 3 apply to the curve of this region. Piedmont is a small residential district with a population of about 7000, and is situated on the hills adjoining Oakland. It contains more open fields, gardens, and lawns than does Oakland, and this undoubtedly accounts for the higher pollen counts. The station in East Oakland was on a hilly section with little vacant land where the prevailing wind was over Oakland and off the Bay. A total curve in a station on

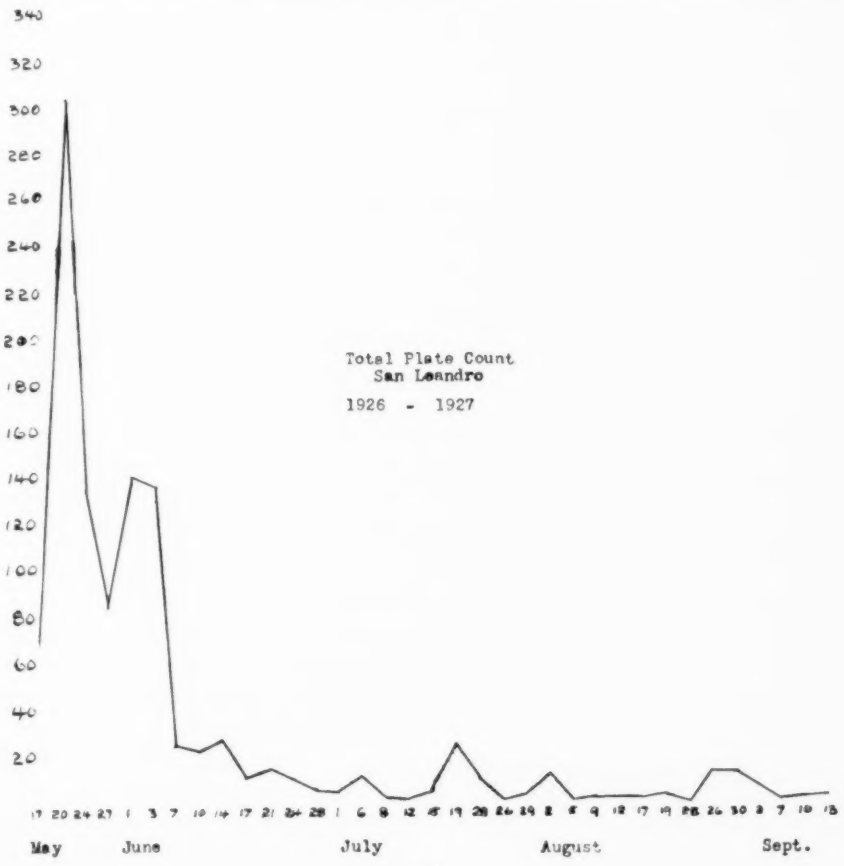


Chart 8.

the outskirts of East Oakland near San Leandro, obtained from May to October, is given in Chart 8, and shows a high peak, similar to those obtained in Richmond. The different types of curves which have been obtained throughout this district demonstrate that a study of the immediate locality in regard to the abundance of the hay fever plants and to the prevailing winds is of great importance in the determination of proper therapy.

This fact is again emphasized by the very low pollen curve in Chart 9 obtained in central Alameda, which is a residential district immediately on the bay and with a prevailing wind off the bay. Again a curve obtained near

the bay in Richmond, as shown in Chart 10, was much lower than that shown in Chart 4 which was obtained two miles inland, where open fields extended to the west. The curve of central Berkeley, shown in Chart 11, is similar to that of East Oakland, or Oakland itself.

CONCLUSIONS FROM ATMOSPHERIC POLLEN STUDIES

1. This constitutes the first record of an intensive study for a whole year of the atmospheric pollen in a large cosmopolitan area.

2. Pollen has been found in the air throughout the year, except on days of constant rain. A few hours of mild, sunny weather, even with rain throughout the rest of the day, was sufficient to bring pollen into the air.

3. Grass pollen is the most common of all found in the air, and has persisted throughout the year. The least grass pollen was present in August and September when most of the grass had dried up, and before the fall rains began. The probable types of grass pollen found during the year will be included under the discussion of the botanic survey of this district.

4. Tree pollen has occurred from January to July. Since it was possible to identify the pollens of most of the trees, as indicated in Table II, these data help in the determination of the pollination season. Pine pollen was found throughout the year, due to the large number of species in this region. No evidence has been obtained, however, that pine pollen causes sensitization. Eucalyptus pollen gives skin reactions on a number of people and probably causes some allergic symptoms. Proximity to trees, to the pollen of which patients are sensitive, must determine the necessity of desensitization. Sycamore pollen was not recognized on any of our slides, probably due to the absence of such trees from the immediate regions surrounding our stations. It is our observation, moreover, that such trees do not pollinate profusely in the East Bay. However, because of the frequency of sensitization to this pollen and the rather common occurrence of such trees, it must be kept in mind in this locality as a cause for allergic symptoms. The same considerations appertain to oak pollen because of the prevalence of oak trees in many regions of this territory. Acacia pollen, though usually carried by insects, has been found on our plates, and it causes definite hay fever and asthma in some patients.

5. Rumex pollen is included with Plantain in Table II because of their marked microscopic similarity. As indicated in Table II, however, we believe that they can be differentiated fairly accurately on the pollen plates. Our deductions are that Rumex pollen is more abundant in March and April, and that Plantain pollen is more abundant during May and June.

6. The relatively small average amounts of Compositae, Amaranth, and Chenopod pollens found in the air of this district, are shown by our data. In the presence of skin reactions and of symptoms occurring at the times of pollination of these pollens, desensitization with them is worthy of trial according to our experience. Local prevalence of any of these weeds would result in a local increase in the atmospheric pollen of such weeds, which would

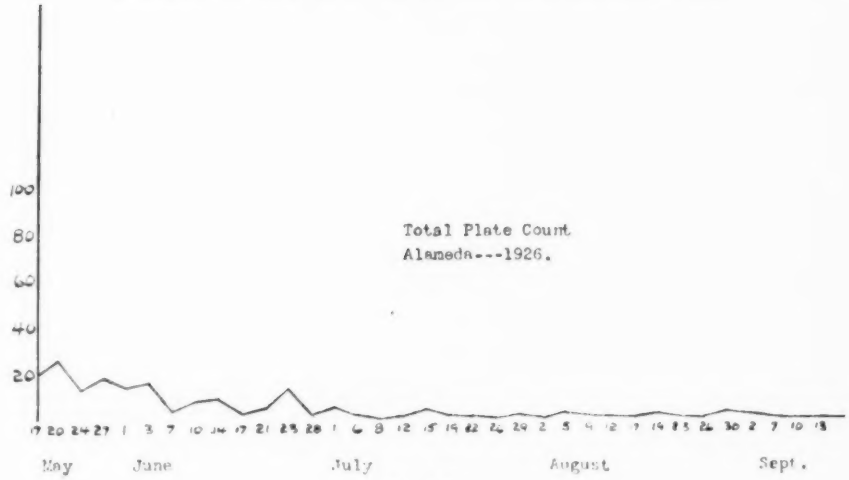


Chart 9.

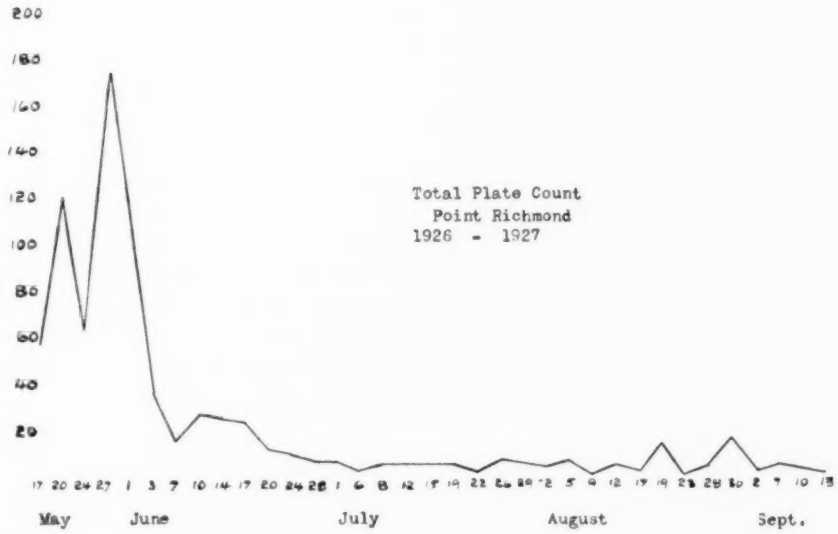


Chart 10.

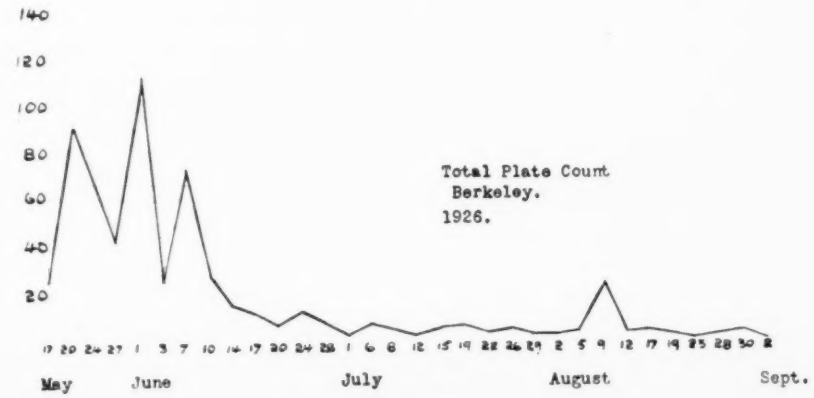


Chart 11.

make such treatment important. The value of a botanic survey of the patient's immediate neighborhood is therefore quite evident, especially in the treatment of refractory pollen-sensitive patients.

7. The definite hay fever and asthma in patients of this district caused by the pollen of weeds, especially of Artemisia and Ambrosia, well emphasize the fact that small amounts of pollen will produce definite allergic symptoms. When from 1 to 5 pollen grains are found in one square inch of plate surface, the patient, in breathing many cubic yards of air, as he does every day, inhales many times this number of pollen grains. However, the number is small in comparison to the maximum counts of Ambrosia pollen reported by Koessler—approximately 900 as compared to our average of 12 to the square inch. This, of course, accounts for the minor significance of our hay fever problem in the fall. I have found many people suffering with mild head congestion, catarrh or bronchitis, and mild hay fever and especially with definite asthma, due to sensitization to fall pollen, instead of the very severe asthma and hay fever encountered in the East and Middle West. Our local problem is likewise different from that of the interior valleys of California, according to my study and treatment of a large number of patients from those regions. The pollens of Ambrosia Psilostachya and the Artemisias and Chenopods are undoubtedly just as productive of sensitization as is the pollen of giant or dwarf ragweed and would cause just as severe symptoms were they found in the air in as large amounts as is similar pollen in the middle western and eastern states.

8. Studies of pollen plates exposed at different levels on the eighteen story City Hall in Oakland confirm Koessler and Durham's conclusion that various species of pollen are evenly distributed throughout the entire air. Table III shows that pollen counts on the average did not vary much on any of the four slides of my own home, emphasizing the even distribution of pollen in the entire air.

BOTANIC SURVEY OF THE EAST BAY REGION OF SAN FRANCISCO BAY IN RELATION TO HAY FEVER AND ASTHMA

My interest in the botanic flora of California, and especially of the East Bay district, began nine years ago when Dr. Grant Selfridge[6, 7] published surveys made of various parts of California by Professor H. M. Hall[5, 7] of the University of California. A survey of the East Bay territory was prepared for me at that time by Professor Hall, and since then I have made constant observations on the flora of all parts of this district, particularly in regard to abundance of distribution and time of pollination of the various species of hay fever-producing plants. During the last year these observations have been extended by an intensive study of this region by a botanist, Miss Weisendanger, and the assembled data are presented in Table V. Professor Hall has checked over our data in regard to frequency of distribution and duration of pollination of the various species included in this survey, for which help I am deeply grateful. The separate districts studied were visited many times during the year to check as accurately as possible on the data presented. The data presented in Table V are arranged in the same manner as those in the

TABLE III

TOTAL POLLEN COUNTS ON VARIOUS EXPOSURES OF A HOUSE IN PIEDMONT

1926	EAST	WEST	NORTH	SOUTH	1927	EAST	WEST	NORTH	SOUTH
Aug. 29			2		Jan. 4	33	7	20	17
" 30			7		" 12	–	15	2	1
" 31		4	–		" 17	1	3	–	–
Sept. 2		1	–		" 21	5	5	–	–
" 7		–	5		" 24	1	–	–	–
" 10		1	–		" 28	20	23	–	–
" 13		1	–		Feb. 4	6	10	–	–
" 21		–	2		" 7	12	7	–	–
" 23		–	–		" 10	52	8	–	–
" 24		3	11		" 14	2	5	–	–
" 25		–	–		" 24	6	13	–	–
" 30		1	–		Mar. 1	3	7	–	–
Oct. 3	20	27	2	3	" 7	70	233	–	–
" 7	8	8	2	7	" 10	13	2	–	–
" 10	9	7	3	3	" 15	40	14	–	–
" 14	2	1	–	3	" 18	54	42	–	–
" 18	3	1	3	1	Apr. 5	3	54	–	–
" 21	–	1	–	3	" 7	55	43	–	–
" 25	2	1	1	15	" 15	11	35	–	–
" 27	4	2	–	1	" 18	6	19	–	–
Nov. 1	1	–	1	–	" 22	5	49	–	–
" 4	7	4	1	4	" 25	2	33	–	–
" 8	–	–	–	2	" 28	30	8	–	–
" 9	–	–	–	13	May 2	9	8	–	–
" 11	1	–	1	1	" 5	5	45	–	–
" 15	8	–	4	3	" 22	1	2	–	–
Dec. 3	2	–	2	6	" 24	6	8	–	–
" 6	8	–	5	2	" 28	20	4	–	–
" 12	2	1	3	–					
" 15	–	–	2	–					
" 20	10	8	2	3					
" 27	3	1	1	3					

excellent survey of Southern California by Piness, Miller and McMinn.[8] This survey of the east shore of San Francisco Bay adds another study to the sectional ones already published by Scheppegrell,[10] Key,[9] Watson and Kibler,[11] Bernton,[12] Templeton,[13] Duke and Durham,[14] Kahn,[15] Balyeat,[16] Phillips,[17] and Waring.[18]

This table indicates that the trees, except for eucalyptus and pine, pollinate during February, March, and April. The relative abundance of each species is shown for the different districts. The importance of tree pollen in hay fever or asthma depends on the proximity to the given trees, the amount of pollen produced, and on the susceptibility of the patient, as indicated by history and skin reactions. This would apply especially to such trees as olive, which are scattered throughout this region and to the pollen of which many patients are markedly sensitive. The same considerations are true of sycamore and oak, which are relatively more common as indicated in the table of distribution.

The important grasses out of the 43 fairly common species included in Table V can be ascertained by a study of the table. Such an analysis will indicate that the grass pollen which is found in the air throughout the year, as indicated in Chart 3, consists largely of Poa annua pollen through the winter months, of Poa annua, Poa pratensis, Bromus hordeaceus, and Festuca pollens during March, April, and early May; of Elymus, Phalaria, and especially Lolium perenne in late May and June; of Lolium perenne and

TABLE IV

PERCENTAGE OF VARIOUS POLLENS IN THE AIR THROUGHOUT A PERIOD OF ONE YEAR, BASED ON 649 PLATE COUNTS MADE AT FROM FOUR TO TEN STATIONS IN THE EAST BAY DISTRICT

Our Findings (East Bay District)			*Dr. Koessler's Findings (Chicago)*	
		PER CENT		PER CENT
Gramineae		50.0	Ragweeds (4 species)	65.3
			Grasses	21.4
Trees—			Chenopod	4.2
Acacia	2.0%		Amaranth	2.1
Pine	8.0%		Dock and Plantain	2.0
Eucalyptus	0.1%		Compositae	1.0
Oak	3.0%		Hop and Hemp	1.0
Juglandaceae	0.2%	27.1	All others	3.0
Palm	2.0%			
Birch	0.4%		Total	100.0
Magnolia	0.4%			
Salix	11.0%			
Rumex and Plantago		11.0		
Other Compositae		4.0		
Artemisia		3.0		
Amaranth and Chenopod		2.0		
Zea Mays		0.6		
Cruciferae		0.2		
Typhaceae		0.1		
Miscellaneous		2.0		
Total		100.0		

Cynodon Dactylon pollen in July, August, September, and October. Local surveys of the patient's immediate environment would be necessary to decide the importance of any other species which might be in unusual abundance and be the cause of symptoms in the individual patient. The treatment of the pollen-sensitive patient with grass pollen antigens which specifically desensitize the patient against pollens to which he is exposed, is, in my mind, productive of the best clinical results.

The importance of the pollens of various weeds is indicated in Table V. Certain pollens, such as Amaranthus retroflexus, are important only when local surveys or pollen plates show that such plants or pollens are common in the patient's living or working vicinity. However, if business or pleasure takes the patient through territory where pollens other than those in this table are found, desensitization to these must be carried out. Although the Artemisia vulgaris is fairly common throughout this whole district, pollen counts do not show that much pollen is in the air of the residential areas. Again Artemisia Californica is abundant all over the hills immediately adjoining, and these hills are gradually being developed into residential tracts. Patients sensitive to either Artemisia or Ambrosia pollen, driving through or working in country or hills where such pollen is common, will develop symptoms which will demand treatment with these pollens.

Patients giving reactions to Chenopod, Atriplex, Rumex, or Plantago pollens, who have symptoms corresponding with the pollinating season, as indicated in the table, should be desensitized with the proper specific antigens. Necessity for Xanthium and Franseria pollen desensitization in this region must be determined by the prevalence of such species in the patient's neighborhood and the severity of skin reactions.

TABLE V
THE MOST IMPORTANT HAY FEVER PLANTS OF THE EAST BAY REGION OF CALIFORNIA

NAME		DURATION OF POLLINATION												DISTRIBUTION BY DISTRICTS						
Bracketed names are plants of most common occurrence *Most important hay fever plants based upon skin reactions.		Each figure "1" represents approximately one week. Italic figures indicate period of most profuse pollination.												Numbers indicate relative amounts in each district. 1—rare 3—common 2—scarce 4—abundant						
Group I Scientific Name	Trees Common Name	January	February	March	April	May	June	July	August	September	October	November	December	Oakland	Piedmont	Berkeley	Alameda	Richmond	EastOakland	San Leandro and Haywards
[Acacia]	Acacia	11	*1111*	*1111*	1111									2	3	2	3	3	3	3
[Acer saccharum]	White Maple			*111*	*111*									2	2	2	2	1	3	2
" negundo	Box Elder		*111*	*1111*	1									1	1	1	1	2	2	2
" saccharium	Silver Maple			*1111*	*111*									1	1	1	1	1	1	1
Alnus rhombifolia	Alder		*111*	*111*										...	2	2	...	...	1	2
Betula alba	White Birch			*111*	*1111*									2	2	3	2	1	2	2
*Juglans Californica	Calif. Black Walnut			11	*1111*	11								1	1	1	1	1	1	1
" regia	Eng. Walnut			*11*	*1111*	1								1	1	1	1	...	1	2
[*Platanus orientalis]	Oriental Sycamore			*11*	*1111*									2	3	2	3	3	2	3
" racemosa	Western Sycamore			11	*1111*									...	...	...	...	...	...	2
Populus deltoidea	Carolina Poplar			*1111*	*111*									1	2	1	1	2	3	2
[Quercus agrifolia]	Coast Live Oak			*111*	*111*									3	4	2	3	...	3	3
" lobata	Valley Oak			*11*	*1111*									...	...	...	...	...	...	3
" dumosa	Scrub Oak			1	*1111*									...	2	2	...	...	...	1
" kelloggii	Black Oak			*11*	*1111*									1	1	1	...	...	2	2
Salix lasiolepis	Arroyo Willow		11	*1111*										1	2	1	2	1	2	3
" Babylonica	Weeping "		11	*1111*										2	1	2	1	...	1	2
[Eucalyptus spp.]	Gum Tree					1111	1111	1111	11					2	3	3	2	3	2	3
[Pinus spp.]	Pine	1111	1111	1111	1111	1111	1111	1111	1111	1111	1111	1111	1111	2	3	3	2	2	3	2
*Olea Europa	Olive					*1111*	*111*							2	2	2	1	1	1	2

TABLE V—Continued

Group II Scientific Name	Grasses Common Name	January	February	March	April	May	June	July	August	September	October	November	December	Oakland	Piedmont	Berkeley	Alameda	Richmond	EastOakland	San Leandro and Haywards
*Agrostis palustris	Red Top					11	1111	1111	1					...	...	2	...	1	...	....
" verticillata	Bent Grass					11	1111	1111	111					1	1	1	1	1	2	2
" exarata	White Top				1	1111	11							...	1	...	...	...	...	....
Avena sativa	Cultivated Oats			11	1111	1111	11							1	1	1	1	2	1	2
[*Avena fatua]	Wild Oats			11	1111	1111	11							2	3	3	2	4	3	4
[Avena barbata]	" "			11	1111	1111	11							2	3	3	3	3	2	4
*Briza minor	Quaking Grass					1111	1111							...	...	1	...	...	1	1
[*Bromus carinatus]	Brome Grass				1111	1111	111							3	3	2	2	3	3	2
[*Bromus hordeaceus]	Soft Cheat			1	1111	1111	11							3	2	2	2	3	3	3
[*Bromus rigidus]	Broncho Grass				111	1111	11							3	4	3	3	3	3	3
" rubens	Red Brome			111	1111	1111	11							1	1	1	1	1	1	1
[*Cynodon dactylon]	Bermuda Grass				111	1111	1111	1111	1111	1111	1111	11		3	1	2	3	3	2	3
*Dactylis glomerata	Orchard Grass				111	1111	1111	1111	1					2	1	1	2	1	1	2
[*Distichlis spicata]	Salt Grass				1	1111	1111	1111	1111	1				2	...	2	3	4	2	2
Bromus maritimus	Seaside Brome Grass					1111	1111	11						...	...	1	1	...	...	....
*Elymus condensatus	Giant Rye Grass					111	1111	1111	11					...	...	1	1	1	1	2
[* " triticoides]	Alkali " "					1111	1111	1111	11					2	3	1	2	3	3	3
Lepturus cylindricus	Thin Tail "					1111	1111	1						...	...	...	...	1	...	....

Bromus uniloides	Rescue Grass					1111	1111								...	...	1	...	...	...	....
[Gastridium ventricosum]	Nit Grass				1111	1111	1111	1111	1111						...	1	...	...	...	1	....
*Festuca myuros	Rattail Fescue				1111	1111									3	4	3	3	4	3	3
" microstachys															2	3	3	...	3	3	2
[*Hordeum murinum]	Farmers' Fox Tail				111	1111	11								4	3	3	3	4	4	4
" nodosum	Meadow Barley				111	1111	111								1	1	1	1	1	1	1
" sativum	Cultivated "				111	1111									2	2	2	2	2	2	3
Koeleria cristata	Koeler's Grass				111	1111	11								...	...	1	...	...	...	....
[Lolium perenne]	Eng. Rye Grass					1111	1111	1111	1111	1111					4	4	4	4	4	4	4
" subulatum	Slender Rye Grass					1111	1111	1111							...	...	...	...	...	1	....
Melica imperfecta	Melic Grass				11	1111									...	1	1	...	...	1	....
*Notholcus lanatus	Velvet Grass				1	1111	1111	1111	1111						1	2	1	1	2	1	2
*Phalaris minor	Small Canary Grass				1	1111	1111								2	2	2	2	2	2	2
[*Phalaris paradoxa]	Gnawed Canary Grass					1111	1111								1	...	1	1	1	2	1
Holcus halepensis	Johnson Grass						1111	1111	1111						...	...	...	...	...	1	1
[*Poa annua]	Walk Grass	1111	1111	1111	1111	1111	1111	1111	1111	1111	1111	1111	1111		4	4	4	4	3	4	3
[*Poa pratensis]	Kentucky Blue Grass				11	1111	1111	1111	11						2	2	2	2	2	2	1
Polypogon monspeliensis	Beard Grass				1	1111	1111	1111	11						1	1	2	2	2	1	2
*Phleum pratense	Timothy					1	1111	111							1	...	...	...	...	...	1
Spartina foliosa	Cord Grass					1111	1111	1111	11						1	...	2	2	3	...	....
Stipa setigera	Bunch Grass					11	1111	1111							...	2	2	...	2	...	2
Triticum vulgare	Wheat				11	1111	111								...	2	1	...	1	1	2
Syntherisma sanguinalis	Crab Grass					11	1111	1111							...	...	1	2	...	1	....
Danthonia Californica	Wild Oat Grass				11	1111	111								1	1	1	...	...	1	....
Echinochloa crusgalli	Barnyard Grass					11	1111	1111							...	...	1	2	...	...	....

TABLE V—Continued

THE MOST IMPORTANT HAY FEVER PLANTS OF THE EAST BAY REGION OF CALIFORNIA

Group III Scientific Name	Weeds Common Name	January	February	March	April	May	June	July	August	September	October	November	December	Oakland	Piedmont	Berkeley	Alameda	Richmond	East Oakland	San Leandro and Haywards
Amaranthus deflexus	.....						1111	1111	1111					3	...	3	3	...	3	3
[*Amaranthus retroflexus]	Rough Pigweed						11	1111	1111	1111	1			1	...	2	2	3	2	4
Amaranthus graecizans	Tumble Weed						11	1111	1111	1111	1			2	...	2	2	2	2	3
[*Ambrosia psilostachya]	Western Ragweed								11	1111	1111	1		2	2	2	2	3	2	2
[*Artemisia Californica]	Coastal Sage-brush							11	1111	1111	111			2	4	3	...	2	3	2
[*Artemisia vulgaris]	Mugwort							1111	1111	1111	1111			3	3	3	3	3	3	3
*Artemisia dracunculus	Tarragon													1	...	1	...	...	...	1
*Artemisia biennis	Wormwood							1111	1111	1111	1111			...	...	2	3	2	...	....
*Artemisia pycnocephala	.....													...	...	...	3	...	...	....
[*Atriplex patula]	Spearscale							11	1111	1111	111			2	...	3	3	3	2	2
" leucophylla	.....					1111	1111	1111	1111	1111				...	...	1	1	...	...	....
[*Chenopodium album]	Lamb's Quarter (White Pigweed)					11	1111	1111	1111	1111	11			3	3	3	3	3	2	3
" ambrosioides	Mexican Tea					11	1111	1111	1111	1111				...	...	...	...	...	...	2
* " murale	Nettle-leaf Goosefoot				11	1111	1111	1111	1111	1111	1111			4	2	4	4	3	3	4
" rubrum	Red Goosefoot													...	...	...	...	...	...	2
[Franseria bipinnitifida]	False Ragweed				11	1111	1111	1111	1111	1111				1	...	2	3	...	...	....
Iva axillaris	Poverty Weed					11	1111	1111						...	...	1	...	2	...	....
[*Plantago lanceolata]	English Plantain				1111	1111	1111	1111	1111	11				4	3	3	3	3	3	3
[*Plantago major]	Common Plantain				1111	1111	1111	1111	1111	11				2	2	2	2	2	2	2
[*Rumex acetosella]	Sheep Sorrel			1	1111	1111	1111	1111	1111	111				3	3	3	3	3	3	3
[* " crispus]	Curly Dock				1111	1111	1111	1111	1111	11				3	3	3	2	3	2	2
[" pulcher]	Fiddle Dock				1111	1111	1111	1111						3	2	3	3	3	2	3
[* " conglomeratus]	Green Dock			1	1111	1111	1111	1111	1					2	2	3	2	2	2	2
[*Salicornia ambigua]	Pickle Weed					1	1111	1111	1111	111				3	...	3	4	4	2	2
Suaeda Californica	Sea Blite									1111	1111			...	...	1	2	2	...	2
Urtica gracilis	Creek Nettle					1111	1111	1111	11					1	2	1	...	1	1	1
" urens	Small Nettle				1111	1111	1111	1111						1	1	1	...	1	1	1
Xanthium canadense	Cocklebur				111	1111	1111	1111	1111					...	...	2	2	2	...	2
[* " spinosum]	Spiny Clotbur					111	1111	1111	1111	11				2	2	3	2	3	2	3
Rumex occidentalis	Western Dock						1111	1111	11					...	...	...	...	2	...	....

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PURPURIC SMALLPOX

Review of Recent Studies*

By Kano Ikeda, M.D., St. Paul, Minn.

PURPURIC smallpox is a rare clinical entity characterized by the total absence or atypical development of the pocks and by a diffuse erythema, and generalized hemorrhagic changes of the body. Its diagnosis is often confusing, and its differentiation from other purpurae extremely difficult. It is always fatal and constitutes by far the smallest group of malignant smallpox. This, in substance, was the experience of the Minnesota epidemic of 1924-1925.

Case A-24-447.[1]—A white man, aged sixty-four years, an orderly in the contagious wards of the hospital, was suddenly taken ill with severe headache and nausea on June 20, 1924. His skin was flushed. Next day he developed severe pain in the right lumbar region and in the upper abdomen which radiated to the right shoulder and to the right groin. On June 22, his face became bright red. His throat was injected. His entire body became covered with a diffuse erythematous rash. There were numerous petechiae and ecchymoses over the abdomen and extremities. Temperature and pulse were normal. Leucocyte count was 11,000. Death occurred on the following day or the fourth day after the onset of illness. No definite clinical diagnosis was made. This occurred at the time when comparatively few cases of smallpox actually existed in Minneapolis and no epidemic was declared in existence in the city. At the coroner's necropsy, aside from the extensive hemorrhagic changes already described and the multiple hemorrhages within the body cavities, no pathology which might account for this sudden termination was found. Death was certified as purpura hemorrhagica; direct cause undetermined.

Some six months later, however, prompted by the experience of the intervening months, a careful microscopic examination of the skin was again made and characteristic early lesions of smallpox was demonstrated.

During the height of the epidemic, not a few patients were seen at the receiving ward of the hospital on whom a diagnosis of measles, scarlet fever, or influenza had been made and who died within from twenty-four to seventy-two hours from purpuric smallpox, indicating that the average practitioner might not be prepared to recognize the condition even during an epidemic.

Similar experiences were reported by Councilman[2] and Litten[3] and more recently by the health authorities of Windsor, Ontario; Detroit, Michigan, and of the Minnesota State Board of Health,[4] all of which illustrate the diagnostic difficulties of this particular type of smallpox.

Twelve hundred and seventy-six cases of smallpox were reported in Minneapolis during the twelve months ending February 28, 1925, with three hundred and thirty-two deaths, or a rate of 26 per cent.

Four hundred and eighty cases came under our hospital care during the same period with two hundred and nineteen deaths, or a rate of 44 per cent.

*Read before the Sixth Annual Convention of the American Society of Clinical Pathologists, Washington, D. C., May 13, 14, and 16, 1927.

From the Pathologic Laboratories of the Minneapolis General Hospital, and St. Luke's Hospital, St. Paul, Minn.

Forty-eight cases, or 10 per cent of the total number of admissions, were diagnosed as purpuric smallpox, all of which died.

The present report is based upon the studies of these forty-eight clinical cases of purpuric smallpox, a large number of the skin lesions being obtained

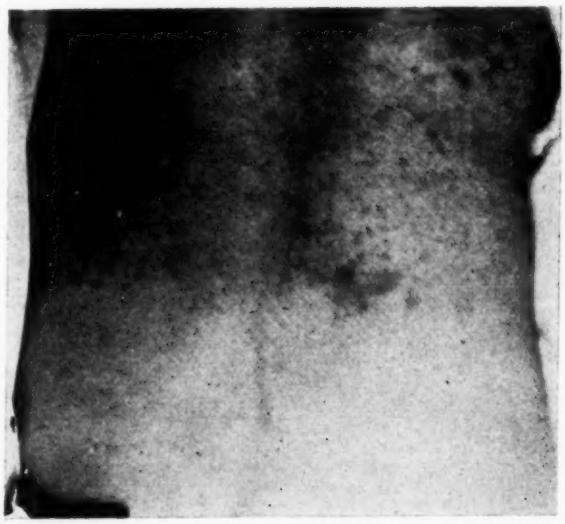

Fig. 1.—Petechial spots in purpuric smallpox.

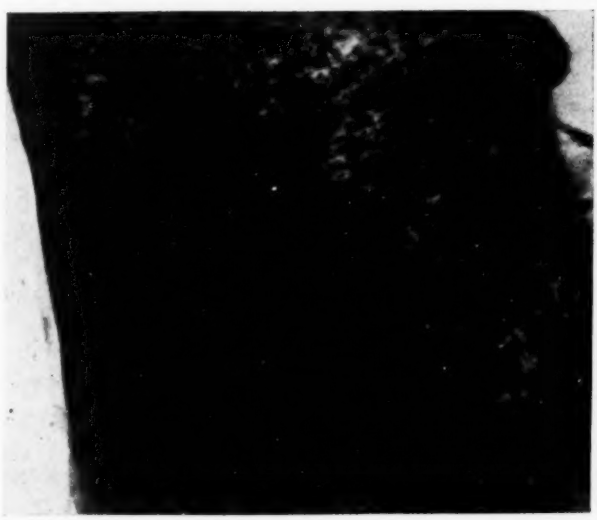

Fig. 2.—Diffuse petechiae ecchymoses and subcutaneous hemorrhages in purpuric smallpox.

by postmortem biopsy and five complete necropsies, as well as upon the similar studies of a large number of cases of pustular smallpox.

Detailed reports on the clinical and hematologic studies of these 48 cases and on the studies of the blood in over 200 cases of smallpox have already been published elsewhere and are, therefore, omitted.[5, 6]

Two distinct clinical types of hemorrhagic smallpox were recognized; namely, the hemorrhagic pustular type and the purpuric type. The latter was again differentiated into the primary form and the secondary form.

Briefly, the hemorrhagic, pustular type was characterized by hemorrhages into the fully developed pustules of confluent or severe pustular smallpox. The mortality in our series of 131 cases was 80.9 per cent. It was probably a local hemorrhagic reaction to the secondary invasion of hemolytic streptococci into the pocks. The organisms were recovered from the lesion, often in pure

Fig. 3.—Early variola degeneration of the epidermis, massive subcutaneous hemorrhages.

culture, in all the cases examined. Typical nonhemorrhagic pustules, on the other hand, were always sterile.

In the purpuric type, the entire clinical phenomena would seem to be explained on the basis of a systemic insult incident with this infection and consisting of a series of true purpuric changes aptly grouped under the term "hemorrhagic diathesis." It was characterized by the conspicuous absence of the pocks and by a diffuse erythema of the skin accompanied, sooner or later, by the widely scattered patchy ecchymoses and punctate subcutaneous hemorrhages over the entire body. They appeared in crops, many became

enlarged and coalesced. They showed no particular areas of predilection. The face and neck showed few petechiae or ecchymoses but were often deeply red or described as "Dusky bluish purple," while the dependent parts showed a deep plum color discoloration (Figs. 1 and 2).

Subconjunctival hemorrhages were a constant and early change noted practically in all cases of purpuric smallpox.

In a group designated as the primary form of which there were 26 cases, no other skin manifestations were observed clinically. In another group of 22 cases, identified as the secondary or late form, in which the cutaneous discolorations were less conspicuous until late in the course of the disease, there were, in addition, a varying number of atypical pocks scattered over the body, either diffusely or more often in limited areas. These pocks were retarded in their development. They were bluish in color, small, flat and

Fig. 4.—Confluent degeneration of the epidermis, from Case A-24-447.

slightly umbilicated, and contained little exudate. More often, they resembled dried vesicles containing little or no fluid. The diffuse purpuric changes took place in the period corresponding to the late vesicular or the early pustular stage, often the case having begun as a usual form of pustular smallpox.

In many instances, mortification of the body, in certain parts, preceded death by many hours; this accentuated the ghastly appearance of the victim to a degree seldom observed in other diseases.

Histologically, the cutaneous lesions were characterized by a diffuse edema in the papillae and the corium, and in most instances, by massive hemorrhages beneath the epidermis. The capillaries were often greatly dilated and filled with blood. Perivascular infiltration of mononuclear cells was noted but not conspicuous. In several cases, the hemorrhages extended deep into the subcutaneous areolar tissue. (Fig. 3.)

The changes in the epidermis were less constant in many of the sections of the most typical gross lesions. The epidermis lying over the most extensively hemorrhagic corium often appeared normal. In others, changes were so early as to be entirely overlooked. More frequently, they were early but

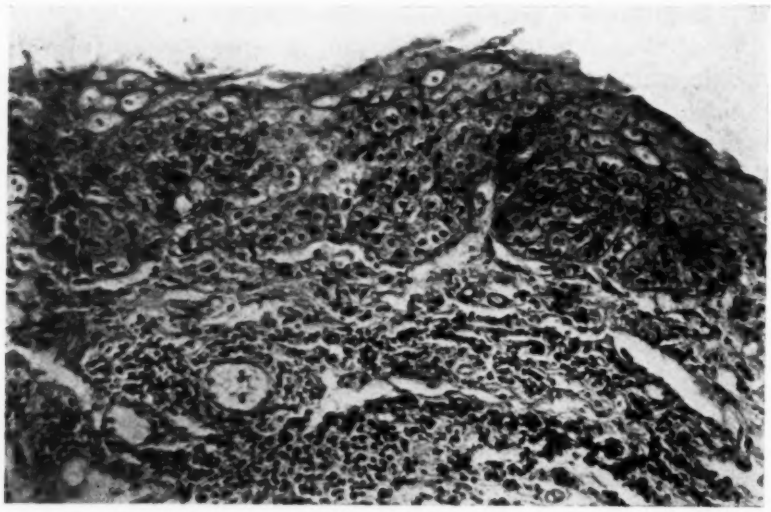

Fig. 5.—Epithelial changes in the pharyngeal mucous membrane.

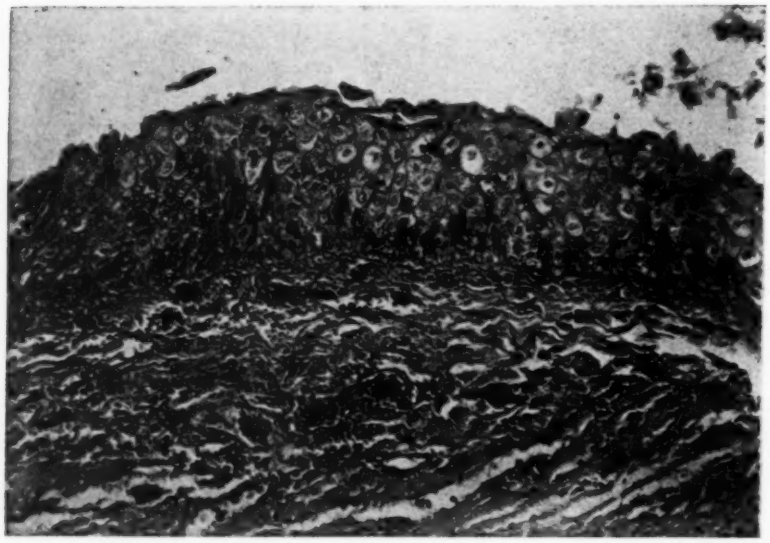

Fig. 6.—Epithelial changes in the tracheal mucous membrane.

sufficiently definite enough to suggest a beginning variola lesion. Again, in a few sections, these changes were so diffuse over a given area as might be designated as confluent (Fig. 4). In no instances, except in the secondary form, however, were these lesions in such a stage of development as to be grossly recognized.

A typical epidermal lesion showed merely the early changes of ordinary pustular smallpox. It consisted of the cloudy, edematous swelling and the blurring of the outline of the cells of the epithelium, the separation of the cells of the basal layer due to the diffuse edema, and areas of the so-called ballooning and reticular degenerations of the epithelial cells, and in some cases, the more specific early vesicle formation.

The two types of degenerations of the epithelial cells were recognized simultaneously; they occurred side by side. Ballooning degeneration was characterized by the metamorphosis of the cells into a perfectly round vacuole-like body with a smooth outline and a darkly staining, shrunken, round or distorted nucleus centrally or peripherally located. In reticular

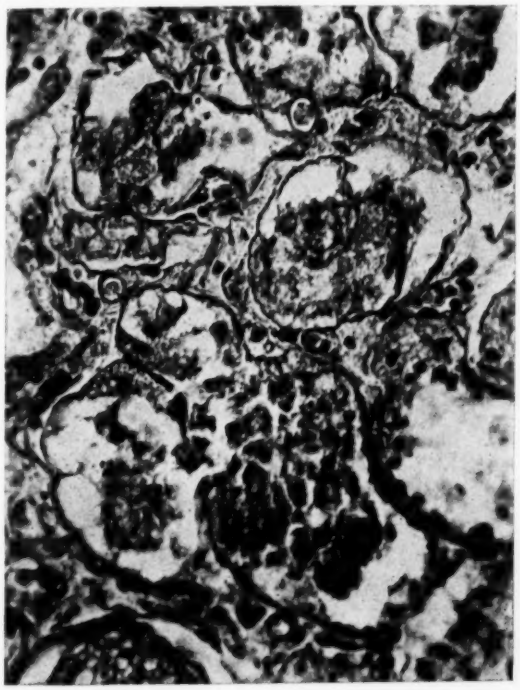

Fig. 7.—High power reproduction of degenerating and exfoliating glands in the submucosa of the respiratory tract.

degeneration, the nucleus became paler and distorted and the cytoplasm swollen and granular. Coalescence of two, three, or more of these cells resulted in a large multinucleated cell or a larger trabeculated vacuole containing cellular debris and other degenerative products. These vacuoles were of various sizes. They again coalesced, forming an extensive reticulated space in the middle layer of the epithelium which constituted an early vesicle.

Hemorrhagic changes in the interior surfaces of the body were even more pronounced than in the skin, obviously because the more delicate mucous membrane was less resistant to the intrinsic degenerative changes as well as to the pressure of the submucosal edema, capillary engorgement, and extravasation.

Thus, the mucosa of the respiratory tract was uniformly swollen and congested and showed extensive injury to the lining epithelium. Deep punctate hemorrhages or larger ecchymoses were observed along the wall. Microscopically, the cutaneous changes already described were duplicated in substantially the identical manner (Figs. 5 and 6) except that the submucosal injuries appeared, as a rule, more intense and further advanced than those of the corium. The epithelium lining the secretory glands beneath the mucosa showed extensive degenerative changes, and the cells were freely exfoliated into the lumina. (Fig. 7.)

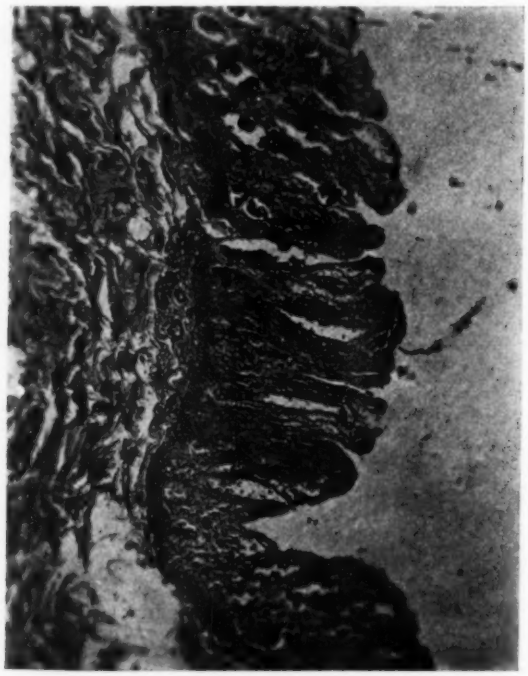

Fig. 8.—Epithelial degeneration of the intestine with an area of necrosis.

The lungs were greatly edematous and hemorrhagic. The gross appearance closely resembled that of hemorrhagic pneumonia of epidemic influenza. Microscopically, edema in varying degrees and extents and a complete exfoliation of the epithelium in the small bronchi were observed. No cellular infiltrates were noted, but the peribroncheal capillary engorgement and free extravasation were present.

The gastrointestinal tract showed a diffuse edema and congestion throughout. Numerous areas of large ecchymoses in the wall and of submucosal hemorrhages were observed all along the tract. The terminal ileum in one case showed a dark bloody discoloration of the wall throughout its entire thickness while in another, the hemorrhages in the colon were more diffuse and confluent; the mucosa was raised in some areas, roughened, and showed a beginning ulceration and sloughing. The lumen contained a large amount of tarry matter. Microscopically, similar degenerative changes of the epi-

thelium leading to necrosis or exfoliation, as well as the diffuse extravasation of blood throughout, were noted. (Fig. 8.)

The heart showed subepicardial and subendocardial hemorrhages. The liver and kidneys also showed hemorrhagic areas beneath the capsules. Microscopically, the liver showed a slight cloudy swelling and numerous wandering mononuclears between the cords and in the portal spaces. (Fig. 9.) The spleen was tense, and the pulp was dark red and firm in consistency. The malpighian corpuscles were not prominent. Hemorrhages were also noted in the mesentery and in the peritoneal wall.

Uterine hemorrhages were noted in all adult females suffering from this type of smallpox. They were interpreted as menstrual by the patient. At

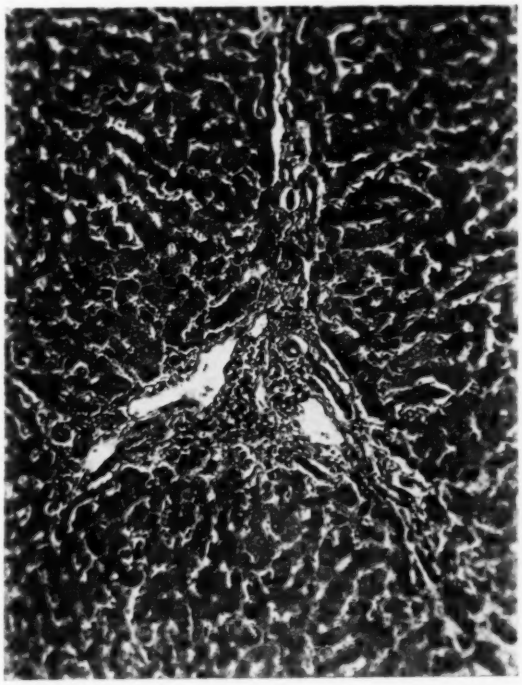

Fig. 9.—Accumulation of lymphocytes in the portal space and their infiltration between the cords of liver cells.

least, in one case, a threatened abortion was seriously considered, and the patient was treated accordingly in a private hospital. Degeneration and exfoliation of the glandular epithelium of the prostate were also present.

Hemorrhages beneath and within the serous membrane lining the body cavities were quite common.

Hemopericardium was noted in one, hemothorax in another, and hemoperitoneum in the third. Two brains were examined. They showed a superficial venous congestion and a diffuse subarachnoid edema. No gross hemorrhages were noted either in the ventricles or within the substance of the brain.

Hemorrhages occurred in the bone marrow in the shaft of adult femur. The yellow marrow was largely replaced by a semigelatinous blood. Microscopically, the field was flooded with blood and showed islands of cellular elements in which normoblasts predominated but which also contained a few myeloid cells. It showed an undoubted hyperplasia.

That the condition presented an extreme degree of bacteriemia was shown by the antemortem demonstration of numerous organisms of the streptococcic group in a simple blood smear obtained from a clean finger tip. (Fig. 10.)

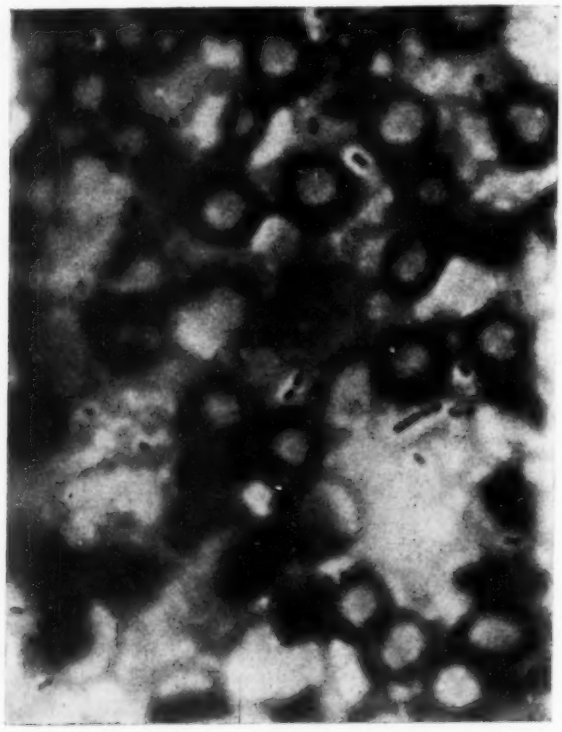

Fig. 10.—An antemortem blood smear showing streptococci.

In one case, direct smears from the cut surfaces of the liver and the spleen, five hours after the death of the patient, showed a large number of streptococci.

Certain characteristic changes observed in the blood of smallpox patients were considered of value, particularly in the prognosis of this disease. In the purpuric type of smallpox, the composite blood picture was not only of a prognostic value, but also by far the most definite and important means at our command of making a diagnosis and differentiation from other purpuric conditions.[6]

The blood findings in purpuric smallpox are briefly summarized as follows:

The Erythrocytes.—The erythrocytes showed a normal count and a corresponding hemoglobin percentage except in a few protracted cases of the sec-

ondary form in which a moderate degree of anemia was evident. A large number of normoblasts and a definite degree of polychromatophilia and basophilic granular degeneration were invariably observed in the circulating blood from the very onset of the disease. These changes often preceded the diffuse cutaneous manifestations.

The Leucocytes.—Rapid disintegration and disappearance of the mature cells of the myeloid origin were noted. Striking metamorphosis of the mature leucocytes in the circulating blood was observed in every case. This consisted of a condensation of lobes of the segmented nucleus into several separate spherical bodies which gradually diminished in number until there remained only a single round body practically devoid of the cytoplasm. (Fig. 11.)

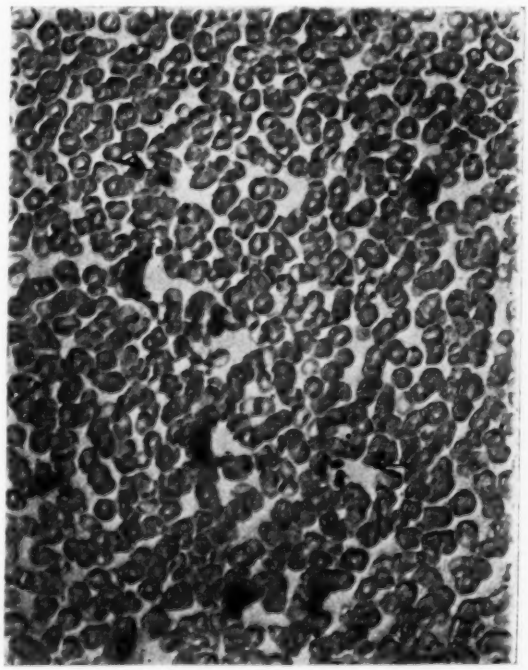

Fig. 11.—Fragmentation and condensation of the nucleus of neutrophilic leucocytes.

The Lymphocytes.—Rapid decline in the number of polymorphous leucocytes was immediately compensated by a rapid increase in lymphocytes which attained more than 90 per cent of all white cells in spite of a persistent and extreme high total count. Many of the lymphocytes were classed as atypical or immature in that they showed many coarse azure granules and basophilic discoloration of the cytoplasm and peculiar lobulation of the nucleus in some, or rather loose chromatin structure of the nucleus with nucleoli in others. Amitosis in some lymphocytes was also observed. In several cases, these changes in the lymphocytes were sufficiently characteristic to be taken for an acute lymphatic leucemia.

The Platelets.—Unlike in pustular smallpox, the initial low platelet count was maintained throughout the course of the disease. It showed a further

decrease until only a few thousands or less were counted in a cubic millimeter. Not a single case was recalled in which the platelet count showed a tendency toward a return to the normal.

These characteristic blood changes were probably by no means specific in the purpuric type of smallpox but rather were interpreted as indicative of a toxemia of the most extreme type, the like of which has seldom been observed in any other disease. This was particularly characterized by the rapid dissolution of the circulating leucocytes, the inhibition of the leucopoietic activity. Diligent studies of the blood in other severe infections and toxic diseases have failed to show the similar alterations.

Practical application of the blood examination in the diagnosis of this type of smallpox and particularly in the differentiation of it from the hemorrhagic pustular type and from acute exanthematous and hemorrhagic diseases, such as scarlet fever, measles, toxic and drug rash, and infectious purpura, was repeatedly found successful during the epidemic when early differential diagnosis of this disease was of the utmost epidemiologic importance.

CONCLUSION

The purpuric type of smallpox presents no anatomic resemblance to the pustular type of this disease. Microscopically, identification may be very difficult in the absence of early variola lesions in the skin.

Diffuse subcutaneous, submucous, and subserous hemorrhages are the most striking feature of this type of small pox. Intense streptococcic bacteriemia is demonstrated in practically all of the cases studied.

The blood picture, while probably not specific, is sufficiently characteristic in every case of purpuric smallpox as to be considered of the prime importance in its diagnosis and its differentiation from other purpurae.

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THE PRESENT STATE OF OUR KNOWLEDGE OF GINGIVITIS*

By Robert A. Keilty, M.D., Washington, D. C.

PURPOSE

THE purpose of this paper is to treat the present state of our knowledge of gingivitis in part as supplied by the literature, which is voluminous and contradictory, and in part by my own experience, which now goes back over a period of fifteen years with quite constant observations. During this time I have presented many papers before different societies but have published very little. Our knowledge of the subject has been a gradual development, and while the last word is still distant, certain facts have been or should be quite well established. At least enough of these facts may be gathered together so that, when the approach to the subject, as suggested by several authors, is fundamentally correct, the main issues will be founded upon certainties, the atmosphere clarified in many respects, and those portions of the subject which are still problematical will be more promptly cleared up.

The dentists, until recently having this field practically to themselves, are quite controversial, and many of the leaders are dogmatically and diametrically opposites. They disagree widely in their conceptions on etiology, and various individual theories on treatment are tenaciously held to. Thus, with the leaders and authors disagreeing so violently, often at times vitriolitic, the rank and file throw up their hands, continue to scale, extract, and use chromic acid.

More recently the medical internist, having become convinced of the importance of oral foci and their relationships to metastatic manifestations, is demanding more from the dentist than a mere tooth extraction or a complete mouth extraction, in the hope that one or more teeth removed may relieve some recognizable chronic infection. With this added stimulus, the laboratory has taken a real interest, and now that it is possible to have the combined opinions of the dentist, the internist, and the clinical pathologist, some light is gradually dawning. I would like especially to thank my coworker, Dr. A. B. Vastine, a dentist and M.D., whose idealism, vision and enthusiasm are second to none and whose inspiration during the past eight years has been a great stimulus.

The purpose of this paper aims further, to discuss the clinical and pathologic types of gingivitis with methods of study and a standardization for reports. A report on five thousand cases with the bacteriologic findings of over four thousand either by smear, dark-field, culture, or section is included. An attempt has been made to show the relationship of various etiologic fac-

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tors with the position and importance of each, as applied to the etiology of gingivitis and its pathologic reactions. Finally some discussion on treatment has been included, rather reluctantly, because in this our greatest advances must be made in the future.

THE PROBLEM

The term "pyorrhea" we have absolutely discarded, as it is misleading. The minute one speaks of pyorrhea, there is an immediate picture of gums full of pus and teeth falling out. It is just as logical to call only those cases of tuberculosis showing advanced cavity, tuberculosis and disregard all miliary types, as it is to speak only of pyorrhea or consumption of the gums, when this is an end stage of much developmental, forerunning pathology. For the same reasons we have discarded the term "Vincent's angina," because it has been so closely associated with so-called "trench mouth" that it cannot be pictured as anything else. All changes in the gums and investing structures of the teeth of an inflammatory nature we have designated under the general term "gingivitis." We should like to insist on this, since the complication of terms, as suggested by some writers, is not only cumbersome but misleading. The term gingivitis may be qualified to fit the pathology of any given case, as acute ulcerative, chronic suppurative, chronic recessive, and so forth.

Gingivitis may be defined as an acute, subacute, and chronic inflammatory reaction in the gums, and soft, teeth investitures, progressing unless checked to a periostitis and osteitis. It is characterized pathologically, and objectively with variations, by redness, edema, swelling, surface granularity, ulceration, suppuration, and the establishment of deep gingival sulci. Terminally loss of teeth ensues. It is characterized *subjectively often by nothing*, at times by extreme pain and tenderness in the gums and jaws, with teeth grinding, and more often by bleeding, bad taste, and foul breath. Indeed, many of the objectionable breaths are due to the gaseous production of gas-forming cocci in gingival pockets. *Thus gingivitis, all forms of inflammatory reactions in the gums, is inflammatory and as such is infectious in origin.* Gingivitis is present, in confirmation of some, in spite of other, widely advertized dental creams and powders, in about 90 per cent of our population in some form or other. This does not mean that 90 per cent of us have pus pockets or are losing our teeth, but it does mean that we have sufficient potential to reach this end-condition, given time enough.

The general problem of gingivitis is closely related to focal infection, and this paper should be considered in conjunction with my views as stated in a companion presentation.[1] Gingivitis, acting as a composite focus of infection or portal of entry for microorganisms of a virulent or low grade character, is one of the most important and frequent sources of infection in the body protective mechanism. The early recognition is thus of added importance, and its complete control one of the greatest prophylactic and preventive situations confronting us today. With the early recognition, eradication, and control of gingivitis, a great many of the chronic manifestations of midlife and later may be forestalled.

HISTORICAL

Pyorrhea, gingivitis, stomatitis, subperiosteal periostitis with subgingivitis and what not, have been discussed in the literature for a century. I feel that the work of Dr. Allen J. Smith in conjunction with M. T. Barrett and reported by the latter in 1914, suggesting the rôle of endameba as an etiologic factor, followed by Bass and Johns and later by the experimental work on focal infection, especially of Rosenow, has done a great deal to focus attention on and advance the general problems of gingivitis. Barrett[2] reported an endameba in all of forty-six cases of suppurative gingivitis, most of which were advanced cases obtained from the wards of "Old Blockley," and while it was not offered as a sole etiologic factor, it was strongly suspected, and emetine locally was advised. Bass and Johns,[3] in November, 1914, were more emphatic as to the etiologic rôle of endameba (85 out of 87 cases), and offered emetine hypodermically as a remedy. Later they published a book on the subject. At the 1914 Christmas meeting of the American Society of Bacteriologists, Smith and Barrett[4] offered a discussion of various endameba described in the mouth up until that time, and definitely suggested Endameba gingivalis (Gros) as the type which is now held. Kofoid and Swezy[5] in 1925 again studied this protozoa in its relation to E. dysenteriae and determined E. gingivalis (Gros) a distinct type from the latter. This difference was presented by Smith and Barrett[4] June, 1915. We are not so sure that E. gingivalis has not a definite relationship to some intestinal endameba thought to have little pathogenicity, and more work is required on this phase of the subject.

Discussion started, war raged, and while not quite so acrimonious at this time, continues nevertheless, but on a friendly debatable ground. As an illustration, in 1920 I read a paper before the Society of Bacteriologists and Pathologists in Washington and received a short, curt discussion, with the statement that in a series of normal mouths in New York school children endameba had been found in a fairly large proportion and, therefore, it was not to be considered pathologic or pathogenic. This closed the whole subject stat, there and then, showing the trend of thought at that time. I never published that paper. In 1921[6] a preliminary report of two hundred cases was published, and this paper is a follow-up of that earlier work.

Hunter, in 1780, emphasized the fact that the seat of trouble was in the bone. Toirac, in 1839, first used the term "pyorrhea," which I think has had its centennial and should be relegated to the archives in favor of the more inclusive term "gingivitis."

In a paper at the Annual Symposium of the American Stomatologic Association in 1926 Asgis[7] speaks of the approach to the study of this disease influencing the writing of various authors mostly along the lines in which their conceptions of etiology bear and, in reviewing the literature, this is especially true. Asgis includes in his paper forty-nine references, developing the subject with forty-nine points of view.

Gottlieb has conceptions of pathology with which I cannot agree,[8] but he has called attention in the formation of the anatomic ditch at the union of the soft structures of the gum and the tooth to the fact that the detachment

normally never reaches the cementoenamel junction,[9] but is always above it. This I think is most important as an objective sign in determining clinically the presence of gingivitis.

Many and varied reports have appeared on findings of Vincent's spirillum and B. fusiformis by smear, but most of these, while advocating them as possible etiologic factors, associate them with definite "trench mouth" and stomatitis. Burns[10] suggests their significance as precursors of pyorrhea and as possible causative agents in such unknown etiologies as anemia.

The work of Rosenow, following his association with Billings, has done much to establish dental foci of infection. His work has dealt mostly with the tooth and its periapical relationship. I hope to show in this paper that all the principles of that work may be applied to gingivitis and that, in proportion, gingivitis per se is many more times a portal of entry primarily for microorganisms. Thus, it is an even greater potential focus of infection than the periapical sources which are not to be minimized in the least in importance.

In the *Medical Journal and Record*[11] a report of the 1926 Symposium on Stomatology reviews the opinion of France, Italy, Poland, Hungary, Germany, England, and America and gives a cross-section of about where the world stands today on gingivitis.

It is quite impossible for me to review completely this literature in this paper, except to call attention to what seems to me to be high lights in my conception of the approach to the problem. In the studies on etiology some speak of pyorrhea as a manifestation of "loss of local resistance" due to systemic diseases, others as the etiology of those systemic diseases; some attempt a relationship to vitamines and food deficiencies, while others assume a rôle of glandular inactivity. Smoking, gingival trauma in mastication, food débris, toxins, mouth breathers, plates and dentures, bridges and crowns, salival inactivity, acid mouth, abnormal calcium phosphates in the saliva producing calculi and tartar, all have their important adherents. Infection as a causative factor is stressed by only a few authors but mentioned by many. When it comes to treatment, the widest variations prevail, from the occasional paper on vaccines condemned by Krumwiede and Pratt in 1915, through papers on general and local measures, to the radical surgery advocated by Neumann.

A research group at the University of California organized in 1921, of which Beckwith and Simonton[12] have published several papers, has developed along the lines which our own work has taken more nearly than any other group so far as I know. In a recent progress report, however, the conclusion is drawn that "their accumulating data appears to indicate that pyorrhea belongs in the category of nutritional diseases * * *. This does not preclude the possibility of the clinical pictures being often and largely dominated by an infectious process * * * superimposed on an initial predisposition of nutritional origin." As the present paper will develop, I have been led to an entirely opposite conclusion, and I hope it is not due to a too persistent attachment to our avenue of approach.

I have said that gingivitis is a focus of infection, and in a recent paper have outlined these conceptions.[1] The importance of this bears repeating.

The gingiva, the seat of inflammation, offers one of the best multiple portals of entry for such microorganisms as the streptococcus to be picked up by the subgingival lymphatics and blood vessels for distribution throughout the body that I know of. In this sense both as portals of entry and as potential focal sources the gingival position is the *most important* in the study of focal infection and control of distant manifestations, such as rheumatism, myocarditis, nephritis, arthritis and so forth. Beckwith, et al,[13] have reported with photomicrographs the proof of the presence of organisms in the subgingival lymphatics, and I have seen them many times, the same tissue being controlled by cultures. Thus it may be an established fact that microorganisms admitted through pathologic gingivitis establish themselves in the subgingival tissue where they act as foci of infection fulfilling two, three, and four of my conceptions as foci.[1]

GINGIVITIS CLINICALLY AND PATHOLOGICALLY

A discussion of what constitutes the normal gingiva and its relationship to the teeth appears in a previous paper.[1] In this paper I have also gone into some detail as to just what gingivitis is clinically and pathologically. Gingivitis divides itself into acute, subacute, and chronic forms based upon its pathologic reactions associated with its clinical course. This classification is influenced by local variations and etiologic gradations only as those are modified in each case after careful clinical and laboratory study. These gradations, modifications, and variations are not only important for properly fixing the type of case for study and teaching but are most important to the individual patient in outlining treatment. Our fundamental conception of gingivitis not only includes the terminal suppurating pus pockets which anybody can see but all the minor degrees of inflammatory changes involving the gums which are now almost universally passed up as normal. Gingivitis is a progressive disease with very small beginnings, and it is only its terminal stage which is the well-recognized condition now known as pyorrhea.

Gingivitis, then, is a nonspecific inflammation of the gums produced by a variety of infecting organisms starting acutely, or more commonly insiduously, and becoming tenaciously chronic. At first the gingivae show nothing more than redness on pressure with a little granularity on the surface and here and there a shallow increase in the anatomic trenches (this stage of the disease is almost always passed over as perfectly normal). The redness signifying gingival reaction continues, and a little exudate appears in the trenches. This is often found only after careful search and sometimes in a clean mouth only at the occlusal surfaces of molars or about a crown. Nevertheless, it is there as a starting point and *given time* will eventually involve the gingivae of all of the teeth. This infection, redness, and exudation goes on to suppuration, ulceration, and deepening of the trenches, and well-marked pockets are formed about one, two or all the teeth. At this stage, ridging of the gums (granulative subgingival fibrosis) occurs with atrophy and recession from the teeth, resulting in exposure of dentine. During this progression the periosteal investiture becomes involved in many cases, eventually terminating in some form of osteitis. I am firmly convinced that

many, not all, of the so-called periapical reaction cysts and periapical pathology without cyst were originally of gingival origin and progression and had little or nothing to do with root canals.

This condition attacks all ages and both sexes. I have frequently seen it in very young children, and Dr. Vastine now has a young girl under treatment, the eruption of whose second teeth is markedly interfered with by nothing more than a severe infectious gingivitis. This is yielding very nicely to treatment. The acute and subacute stages are by far most commonly seen in young adult life with slow progression on to middle life, when ulceration and more advanced changes are to be looked for. The very advanced chronic cases which are seen now and then in young life are much more frequently in the forty to fifty decade and about the time most of the teeth are lost for one reason or another. It is to the prevention of this great sacrifice of teeth that our future work must lead us, and indeed we have the means at hand now to accomplish this except that it is somewhat complicated. Neglected and dirty mouths are more apt to have advanced changes, but not in every case. Some of the apparently dirty mouths will be relatively free from infection and advanced gingival changes, while, vice versa, some of the cleanest mouths in which oral hygiene is religiously carried out will harbor virulent, tenacious infection leading to advanced gingival changes. Every case is a careful study unto itself, as to history, symptomatology, clinical appearance, distant manifestations, types of and quantitative infection present and individual reaction to treatment. Gingivitis is an entity only in so far as the gums are involved; it is complex in its course, appearance, etiology, and treatment, and as soon as this complexity is understood and accepted, the tangle of the problem presented today will be decidedly cleared up. The recognition, course, appearance, and etiology are relatively simple, but the treatment is much more baffling. This, however, relying on the correctness of the other studies, is now well advanced when certain procedures are carried out and bids fair soon to be completely straightened out.

REPORT ON 5,000 CASES OF GINGIVITIS

This paper includes the report of 4,347 individual cases where the gingivae have been examined bacteriologically by smear, dark-field, culture, or section. These cases do not belong to a group survey but are the routine cases carefully studied presenting themselves over a period of years. All have had smears, a few dark-fields, many cultures, and a few sections. With cases not included in this group, we have seen well over five thousand which we believe should be a number large enough from which conclusions may be drawn. Statistics mean very little ordinarily, but in large groups they at least point to facts. The same relative methods have been used throughout the study, but necessarily experience has brought improvements, and I desire to offer a standardized method for the examination of all gingival cases. This examination, where possible, should be carried out by one person in the laboratory who will have acquired a uniform gradation of amounts. This is quite important in recommending treatment, since the success of the latter depends almost entirely upon the accuracy of the former. If the patient is taken into the

confidence, as he should be in order to gain his cooperation, the improvement as measured by gradation is a good aid to his morale.

The mouth is surveyed generally, and the physical condition briefly noted. In the group study of oral foci of infection this first examination should be quite complete, and while it helps to have the report of the laryngologist and dentist, the examination should not be confined entirely to areas which they may have pointed out. Cooperation in this respect helps indeed. It is not necessary to use the dark-field for examination routinely, as suggested by some, and after a few hundred cases have been examined for familiarity, the dark-field should be reserved for blood luetic serologic positive cases where the question of Treponema pallidum is raised. I have only seen Treponema pallidum once in gingival exudates, but my group has not included many active secondary syphilities.

After the entire gingivae have been surveyed, expose the gums with a wooden tongue depressor moistened in water (so that it does not stick) and obtain material from all possible sources in the gingival sulci both upper and lower. A 22-gauge chrome wire, small loop slightly bent, is most suitable. Platinum wire is too soft and instruments are too stiff; chrome wire is preferred. Wherever the gingival trench is deep the loop should be worked in and out until gross material is obtained. I have obtained positive findings on several occasions, following a negative report, by attention to the detail of obtaining the exudate. This method, if carried out gently, will hardly annoy the patients, many of whom are nervous expecting to be hurt. It is not necessary to flame the loop between different situations examined, since any transfer from one pocket to another does not mean anything in the mouth. After the first flaming, which the patient may see, assure him that the loop is not hot. Care should be taken not to get hemorrhage, since red blood cells in large numbers interfere with the subsequent examination of the slide.

Transfer the materials obtained to a large drop of physiologic salt solution 0.85 per cent, on a microscopic slide, evenly mix with the loop, and cover with a square cover slip. Water, of course, is not to be used. In the summertime the slides do not need to be heated, but if the room is cold and it is winter, the slide should be gently heated over the top of the Bunsen flame. A warm stage may be used, but this is not absolutely essential. The slides, however, *must not be cold.* Examine under the high dry power, 4 mm. objective; as a routine twenty fields should be looked at; more may be, but certainly no less. This will give one a relatively quantitative idea of each group sought and of the preponderance of one over the other. If this quantitative estimation is reported in grades of 1 plus to 4 plus, it will be a great help in outlining and carrying out treatments. Where any type is not present we use N.D., not demonstrable. One plus means present but only a very few, for example one endameba seen in twenty fields or an occasional spironema or pus cell; 2 plus and 3 plus mean increasing degrees, for example one or two endamebae in every two or three fields; 4 plus means large quantities as two or three endamebae in most every field, spironema by the millions or heavy exudate of pus.

After the slide has been carefully examined in the fresh, the cover slip is gently drawn across the slide, the latter is fixed either by heat or by a mercurial fluid and stained by any method preferred. A good Gram stain is perfectly satisfactory; Giemsa, carbol fuchsin weak alone, Wright's stain, Hiss's capsule stain, plain methylene blue, all give good fields. For endameba we prefer a fixation by Schaudinn's mercurial fluid and a stain by Giemsa or iron-hematoxylin. It is believed that the fresh smear examined by the 4 mm. objective gives the very best examination showing all factors to the best possible advantage. The stained smears help out in critical study, and in using the Gram stain, prevailing types of bacteria may be tentatively enumerated, as for example, pneumococcus, streptococcus, B. coli, etc.

The important features to be searched for in order of importance are the spironema, fusiform bacilli, Endameba gingivalis (Gros), bacteria as a group, cocci and bacilli motile and nonmotile, pus cells, red blood cells, desquamated epithelium, and mycelial threads under the general term leptothrix. Unusual findings, such as yeasts, protozoa other than the ameba, crystals, débris, food particles should be noted at the end of the report.

Where cultures are made to determine specifically the bacterial flora or to study in detail a predominating organism, loops are obtained directly from the gingival sulci and planted on appropriate media. The media are warmed in the incubator and immediately incubated. As a routine we use rabbit, guinea pig, sheep, or human blood agar plates (very light blood) streak planting, autogenous blood agar plates, autogenous blood the method of Heist, plain broth, sugar broth, ascitic fluid, and anaerobic cultures. The latter are prepared by inoculating a plain agar slant and stab, pouring a tube of plain broth, glucose broth, ascitic fluid, whole blood, or tissue and covering the top with a good paraffin layer. Special medium, of course, may be added for special purposes. It is not essential to culture mouths routinely unless one has a large laboratory force, but in given cases not responding to treatment a chemotherapeusis may be worked out by autogenous cultures.* It is not practical, of course, to section gingivae routinely, but this should always be done after culturing operative bits of tissue. For study, of course, small bits of tissue may be removed by biopsy or taken at autopsy.

Referring to Table I, which represents the fresh smear examinations, it will be found that spironema appeared 3,778 times or 96.4 per cent, the endameba appeared 2,809 times or 71.6 per cent, the spironema and endameba were present at the same time 2,668 times or 68.0 per cent The spironema appeared alone 1,110 times or 28.2 per cent; the endameba appeared alone 141 times or 3.6 per cent. There were 428 cases which had either no clinical evidence of gingivitis or at least very slight evidence in which neither the spironema nor the endameba could be demonstrated. These include the control negatives, and there was not a single case of clinical gingivitis in which one or the other of these organisms could not be demonstrated.

The 2,613 or 66.6 per cent of the smears showing bacteria represent those cases having outstanding clumps and masses of bacteria growing in the

*Since this paper was presented I am routinely culturing all cases and I would advise at least a blood agar plate be studied for each gingival flora.

TABLE I

INCIDENCE OF OCCURRENCE OF DIFFERENT ORGANISMS

	NUMBER	PER CENT
Total number of cases	5,000	
Number of cases recorded	4,347	
Number of cases of gingivitis	3,919	90.2
Number of cases of normal	428	9.8
Spironema present	3,778	96.4
Endameba gingivalis present	2,809	71.6
Endameba and spironema present in same case	2,668	68.0
Pus cells present	3,919	100.0
Bacteria present	2,613	66.6
Fusiform bacillus present	2,211	56.4
Spironema present alone	1,110	28.2
Endameba present alone	141	3.6
Leptothrix present	2,082	53.1
Trichomonas present	1	0.02
Cercomonas present	48	1.2
Balantidium present	2	0.04

gingival sulci. The 33.3 per cent remaining, of course, were not sterile, but the bacteria were not present at least in smears in sufficient quantities to be noted. Indeed, in smear, normal mouths are surprisingly free from everything. It is frequently very difficult to get even enough to look at. On the other hand, advanced gingivitis has so much that the smears may easily be made too thick to be viewed.

The fusiform bacillus, that is, a large, slightly curved fusiform bacterium, was noted 2,211 times or 56.4 per cent. For some time we have not regarded the relationship between this bacillus and spironema as a symbiosis but have considered that they have a closer bearing. Their position as regarded will be discussed later on. We believe they should be noted where they are present in the absence of spironema, but cannot give any figure on this relationship at this time.

Table II points out the gradations of spironema and endameba based on the standardization previously outlined. In the case of spironema the occurrences as 1 plus, 2 plus, and 3 plus are about the same while the 4 plus is decidedly lower in number. In the case of endameba there is a marked difference between the occurrence of 1 plus and 4 plus. I think these figures are the result of normal etiologic incidence, but I also believe they are reduced in the higher degree by general mechanical oral hygiene as practiced today. While the tooth pastes and powders as used with brushes have no preventive or curative powers in regard to gingivitis, they do help in a general clean-up, and in this way reduce the severity of gingival infections.

The table shows 2,082 cases or 53.1 per cent with outstanding mycelial threads or leptothrix accumulations. We are not in a positive position on this organism and its relationship will be discussed later. The yeasts and fungi are not listed, but their incidence of occurrence is very low, and they have no direct etiologic bearing.

The few protozoa other than endameba were noted and cited for appropriate treatment, but they play practically no part as general etiologic factors in gingivitis. Where present in a given case their activity may irritate and open up portals of entry, and in some cases their persistence is tenacious in

TABLE II

GRADATIONS OF POSITIVES IN NUMBERS PER 20 FIELDS OF EACH EXAMINED

	NUMBER	PER CENT
Spironema 1 plus	1,205	31.6
Spironema 2 plus	849	23.2
Spironema 3 plus	1,212	31.7
Spironema 4 plus	537	13.4
Endameba 1 plus	1,862	65.0
Endameba 2 plus	574	20.1
Endameba 3 plus	322	11.3
Endameba 4 plus	90	3.1

spite of treatment. Hinshaw[14] reported three cases Trichomonas buccalis out of 64 cases cultured or 4.6 per cent. This is considerably higher than our own incidence but not enough to influence the general conclusions in any way.

One hundred per cent showed the presence of pus (that is exudative white blood cells dead or alive in sufficient numbers to be called pus). This represents another point in the inflammatory nature of every case of gingivitis. At the same time, of the 428 cases called normal, 145 or 33.8 per cent had some pus while 283 or 66.2 per cent showed absolutely nothing.

As has already been stated the smear reports in this paper are based mainly on fresh smears examined as recorded. The stained smears were not noted separately, indeed they are no longer carried out routinely and are now used only to bring out certain special features as already described. It is advisable to check the fresh smears with stained preparations until thorough familiarity with the fresh is obtained.

In the examination we are in the habit of first determining the presence of endameba by a search of at least twenty fields and noting the number per field. They are readily found and have many definite characteristics. The important feature of the fresh smear is the movement of endameba which makes them definite. We next determine the presence of spironema noting number, shape, size, and motility; next the relative number and clumping of bacteria and pus cells and any predominating bacteria. In the latter search, fusiform bacilli and leptothrix are included. Finally we are on the lookout for yeasts (we think that routine stained specimens would show a higher incidence of occurrence, but at least for the time being they are relatively unimportant), protozoa other than endameba and anything unusual. This takes in all about fifty fields in the study.

Cultures from gingival sulci show a wide variety of organisms both aerobes and anaerobes. While this variation is marked in a large number of cases, the flora of a certain mouth is relatively constant when it is kept under cultural control from day to day or month to month. Unlike some lesions of the nose and mouth, in which a single organism either develops at the expense of others or in its development becomes repellent to others and the cultures are characteristically single organisms, the gingival sulci as a rule give a very mixed flora. Three, four or more different species is the rule. The coccal group predominates and anaerobes are always present. In the complete study of a given case where clinical signs continue in spite of treatment, where control cultures are advisable before going into deeper structures, or where

a flora is desired for the preparation of a vaccine, material from gingival pockets should be planted on several different types of media and careful fishing and pure culture isolation carried out. This paper is now too long and cannot include details of cultural studies, but suffice it to say that the gingival bacterial flora as a group entity plays an etiologic rôle in gingivitis and a much more important one in the secondary deeper tissue invasion with the development of focal areas of infection.

The sections of gingivae so far studied are quite uniform in their appearance. We are not including here the more complex pathology of the different types of so-called periapical cysts and periapical pathology. We have a number of jaws removed at autopsy including gingivae, teeth, and bone which will be the subject of a separate report. The normal gingiva shows very little other than the epithelial covering the loose connective tissue and lymph spaces with thin-walled blood vessels and a few submucous racemose glands. The latter are not numerous and are rather rudimentary in type. When the gingivae are the seat of inflammatory changes, round-celled infiltration of the wandering lymphocytic and plasma cell forms predominate. The polymorphonuclears are scattered, and occasionally definite small abscesses are to be seen. Where the process is advanced, surface ulceration is present with its line of necrosis, and often against this, marked infiltrating leucocytic lines of demarcation are made out. As the process advances and in the more chronic cases of long duration, much irregular fibrogenetic tissue with strands of old hyalinized bands are present. In the midst of this tissue microorganisms are readily demonstrable by appropriate staining, as has been shown by Beckwith, et al.[13] By careful technic these organisms can be cultured, and the plates rarely show more than two types, and more often a single organism is obtained. This will always bear a direct relationship to the same organism which can be cultured from the gingival sulci of the same case before its surgical removal. Some form of coccus is the predominating type.

Twelve cases were studied every day for thirty days with a daily comparison of results. Eight of these cases were gingivitis of various stages and four were normal controls. They were studied clinically and by smear and culture. They were submitted to recorded control treatments and minute detail as to any influencing factors were recorded. The persistent and tenacious qualities of the various infecting factors were striking, but just as striking was the improvement both clinically and bacteriologically under treatment. The limitations, however, of our present knowledge of efficient remedies was quite apparent from this study. The periodicity of infection was partially established, and at least a tentative program was mapped out for the necessity and times of continued treatments. From the cultures of this group it was found that all sizes, shapes, and activities of spirilliform organisms could be obtained, and we now believe that they all belong to the one group spironema. Subject to further proof it is our opinion that the so-called fusiform bacilli are really mycelial threads segmented from a parent leptothrix body which are both gram-positive and gram-negative according to their stages, which are both motile and nonmotile also according to their stages and which in the motile form angulate and become spirals during a

cycle of their development. It is very difficult to grow these organisms, at least in our hands, but from a composite study of a large number of cultures, again subject to proof, we believe the spiral organisms present in such large numbers in so many mouths are stages, through motile and nonmotile bacilli which are now known as B. fusiformis and are now generally considered in symbiotic relationship, of a parent fixed mycelial or leptothrix grouping of the so-called higher forms of bacteria.

The changes in H-ion concentration of the exudates of gingival sulci as compared with saliva were carried out by the micro method, using the LaMotte colorimetric set-up. In the group the twelve cases were included and 256 observations were made. This number is entirely too small to draw definite conclusions from, but the figures are rather striking. The gingival exudates are quite acid and vary somewhat under different conditions, probably due to the cultural influences of acid-forming bacteria. The exudates are decidedly more acid than mouth salivas. The average P_H of a gingival sulcus, the seat of gingivitis, is 5.72, with a range of 5.4 to 5.8, the lowest 4.6 and the highest 6.1.

THE RELATION OF THE BACTERIOLOGIC FLORA TO GINGIVITIS

The bacteriologic flora of gingival sulci in relation to periapical cultures is, of course, complex but is apparently quite constant and persistent as far as each mouth is concerned. That is, there is not much change in types of the organisms from day to day. The predominating cocci, Staphylococcus hemolytic and nonhemolytic, aerobic and facultative anaerobic, gas-producing and acid-forming, Streptococcus pyogenes, hemolytic and nonhemolytic and the large group of B. hoffmanii are quite constantly present in all mouths. The relationship of this flora or at least of one or two characteristic organisms, to subgingival areas, deep periapical cysts, root canals and infectious osseous cystic mandibular osteitis and sinus involvement are striking, and in a given case the same organisms can be isolated from the gingival as well as from the deeper sources. Of course, there come to one's mind immediately questions of contamination. Let me say here, when one first starts to work on this problem bacteriologically technical difficulties beset one at every hand, but with experience and a complete knowledge of working conditions contaminations can be completely and entirely ruled out and one's results can at least keep one's own mind at rest. The secret of success is scrupulous attention to technical detail and plenty of checking. The only other factor is experience. It was from these bacteriologic findings and checks that we firmly established, at least in our own minds, the fact that gingivitis is so often a multiple form of infection and of much greater importance than root canals in establishing deeper and more potent foci. When this fact was realized and applied to clinical observation and treatment, the results obtained improved markedly and have been most gratifying and confirmatory.

The bacteriologic flora in its relationship to the gingival pathology is worth considerable thought, and upon this relationship hinges the relative importance of each of the organisms enumerated and the parts they play. Assuming the gingival sulci contain a varied flora, is it fair to assume that any

one organism is more important than any other? What proofs are we able to set forth as to the importance of each? In the first place the cultural characteristics of all organisms found must be fully known before any animal experimentation can be successfully made and Koch's postulates fulfilled. It is said that spironema for instance, grows anaerobically, but to accomplish this is extremely difficult. The endamebae have been grown, but to get rich cultures easily is almost impossible at the present state of our knowledge. Until these two organisms are more thoroughly mastered, we feel that any animal work would be incomplete. In order then to give a relative importance to these different bacteria, we must turn first, to the character of the pathology, and second, to the results of chemotherapeutic attacks directed at each with a careful notation of good and bad changes obtained.

The pathology suggests general chronic infection, that is, small doses constantly received with now and then a sharp flareup with suppuration. Endameba has never been found in the gingival tissue. We have looked for it often and still feel that at times it must actually invade the tissue although this is not its habit. Spironema has never been successfully demonstrated in the tissue, but more painstaking search must be made for this. The other groups of organisms have been demonstrated both by section and by culture in the tissue, and the tissue shows pathologic evidence of reaction against these organisms. Endameba, spironema and organisms are found more or less constantly in gingival sulci showing evidence of gingivitis. It is therefore fair, and we believe reasonable to attach the proper etiologic significance to these organisms in so far as our present state of knowledge will allow.

The spironema under most conditions in the mouth are extremely active. They are actually belligerent against cells, they have a rapid mechanical corkscrew movement which is directed actually in mass attack so that they are able mechanically and violently to shake a pus cell. Multiply this action by the billions which are present in gingival sulci and mechanically then, they are the finest kind of a stimulus to set up traumatic reaction. This is entirely aside from any toxic action which they may possess against cells.

The endameba is much slower in its action but just as powerful. Mechanically it is able to wedge itself in and between cells and débris and reminds one of the relative strength of an elephant in ordinary underbrush. On the other hand it is quite likely to have an enzymic, digestive toxin, and its powers of phagocytosis are well known. There is doubt in the minds of some, but they need be troubled no longer if they will but observe that an endameba will engulf a red blood cell. We have seen this on numerous occasions. It was first seen by us in a preparation of Dr. Allen J. Smith's, and since on several occasions we have seen this repeated. The red blood cell is attracted to the surface of the endameba as though by a potential surface tension. It is not actually pulled in by the pseudopods but seems to melt into the body of the endameba which is always in strong activity when this is accomplished. It is a matter of many minutes, as many as twenty, before the red blood cell is actually within the body of the endameba, but once inside its identity is rapidly lost. It seems to be quickly consumed as though by solution. We have spent many hours watching the movements of

endameba, and while one in activity does many queer things, all movements except the digesting of bacteria and red blood cells seem to be absolutely purposeless. This protozoa is composed of a very delicate chemical mechanism, and the least change of the surrounding medium throws it into dissolution. The endamebae then not actually in the tissue but in the gingival sulci are strong mechanical factors and traumatic stimuli for inflammatory reactions.

With the powerful initiating stimulus of both endameba and spironema setting up and continuing an inflammatory reaction, the smaller but more powerful bacteria, many of which are not only motile but dart across the field so fast that they cannot be followed, use the gingival sulci as a portal of entry to the subgingival, periodontal, periosteal and osteal tissue and continue a more virulent and in a way a more important reaction. This leads to tissue pathology, extensive gingival inflammation, multiple foci of infection and from these, organisms are picked up by the lymphatics and the blood and carried to distant and varied parts of the body. This is a slow process, an intermittent process, and mostly, for which we can be thankful, in very small doses. Such bacteria, lodging in distant parts of the body, set up reactions characteristic to their type, influenced by their selective activities, and there is seen the varied symptomatology of what we have spoken of as distant manifestations of oral dental foci of infection.

One of the other factors may be present in a mouth; they help to prepare the field and continue as an irritant, in order that the bacteria may do their actual fighting work. When it is remembered that all the bacteria want is a place to live and some food, and when they find such excellent conditions in gingival sulci for cultural elegance, is it any wonder that gingivitis is so prevalent. These other factors, what Vastine speaks of as the negative phases of the subject, are lack of dental oral hygiene, uselessness of dental creams and powders from a chemotherapeutic standpoint, inefficiency of the toothbrush method in reaching gingival sulci, the mechanics of crowns, bridges, plates and dentures, cavities and dental caries, calcification of gingival exudates, traumatism of chewing, accumulation of foodstuffs in and between teeth, sugars, proteins, and the intravital relation of systemic diseases. These and maybe others play a part in allowing gingivitis to start or in helping to continue it and in that sense are contributory factors, but in no sense are they to be considered prime etiologic factors. They are most important, however, in accomplishing a final cleanup when it comes to treatment.

PRINCIPLES OF TREATMENT IN GINGIVITIS

This paper is now entirely too long to include treatment, but the principles as worked out by Vastine in complete cooperation with the laboratory must be at least discussed. In the first place the bacterial flora of the gingivae with gradations of positives must be studied and used with frequent checkup for all subsequent treatment. It is perfectly proper for the laboratory to suggest types and dosage of chemotherapeutic measures. *The successful treatment does not depend upon any one or two procedures, methods, or drugs but a mass attack using every available method against conditions found in*

the individual mouth. There is no one specific cause for gingivitis and there is no one specific treatment. After all possible data as to etiologic factors have been obtained in a given case, appropriate methods of correction are then to be carried out. If cavities and caries are present, they must be corrected. If bridges, crowns, etc., are wrong they must be corrected (we have seen many beautiful mechanical arrangements which it was absolutely necessary to sacrifice before a given case could be cleaned up). This is a matter of much education, but it might as well be realized sooner than later. If there are any gross collections of so-called tartar or calculus which are traumatic stimuli, they are to be removed, but the general process of scaling, with injury to soft tissues and especially to the tooth, is absolutely condemned and the general practice of scaling might as well stop right now. The same thing holds good with all the various mandril polishers, stones, and wheels which do more damage than good. Every injury to soft tissue and the tooth must be repaired. The tooth has little or no regenerating power; injury to it leads to caries and injury to the soft tissues leads to or increases the chances of portal of entry for microorganisms. The dentists as a whole are, of course, antagonistic to these statements, but that is due to the teaching in our dental schools today, and to the striking lack of knowledge of fundamental pathology. The crying need is a general cooperation in the entire study of this group. We have tried to show the relationship between gingivitis and deeper focal areas and, of course, the cleanup of both areas must go hand in hand. Any other pathologic changes in the nose and mouth, such as tonsils, or sinuses which are just as important, must have an absolutely cooperative cleanup. When all contributory factors have been recognized and means taken to control them, local chemotherapeutic measures, started at the same time or before some parts of the cleanup, as for example before dental foci are touched, should be vigorously carried out. Some patients may require treatments once a week, some once a day, some every two hours all depending upon the clinical characteristics and the virulence of the infecting organisms. Some may clear up promptly, and some may require treatment for months. After a general cleanup a return checkup should be carried on about once a month. The most severe cases after they are under control may be kept so by a treatment varying from once every two to three weeks to two to three months. So far as our chemotherapeutic knowledge goes today, there is nothing which will clean these cases up and keep them clean forever. They all tend to recur, and the very best that can be hoped for is control. This, however, fortunately can be carried on with almost one hundred per cent of assurance if—and so far as we know only if—all the conditions enumerated are established and maintained.

The therapeutic measures are many, but the aniline dyes, arsphenamine, emetine, Dakins' solution, hot water irrigations, sodium perborate, hydrogen peroxide, combined in some combination to fit the bacteriologic flora are the best remedies we have found so far. Mouth washes are valuable merely as cleansers, and any tooth powder or paste is good for cleansing reasons; none of them have any influence directly on the specific factors of gingivitis.

The method for administering various drugs is most important, and that

advocated by Vastine is the most direct and efficient so far devised. The use of the rubber cup gets the drug in the dilution which is best suited directly to the bacteria in the gingival sulci without injury to the tissues. Swabbing, needles, instruments, syringes, pressure devices have all been tested out, and none of these have the efficiency of the small rubber cup. The length of time, the concentration, and the nonirritant chemical character of the drug are all very important as is the interval between treatments.

We are opposed to all operative procedures on the gums, that is in the treatment of gingivitis per se, except in special cases, for example, where one pocket is almost a sinus and a small incision would facilitate drainage or the application of the treatment.

SUMMARY AND CONCLUSIONS

1. The problem of gingivitis is one of the largest and most important phases of medicine as it stands today. It is far-reaching in that over 90 per cent of our population have it in some form or another. Its controversial stage involves mainly the dentists while the medical men are realizing its importance and demanding a more careful attention to it.

2. The term "pyorrhea," representing as it does the end-stage of a disease, should be immediately discarded for a more inclusive term. Gingivitis qualified by acute, subacute, or chronic is suggested, since the disease is in all stages an inflammation of the gingivae.

3. The disease in the majority of cases goes unrecognized in the early stages, runs a long-continued course, finally terminating in pockets, destructions, recessions, and loss of teeth. It is one and the same thing in different degrees of severity and stages of advancement.

4. A plea for early recognition and adequate prophylaxis is made, and if carried out, many of the terminal changes will be avoided.

5. The gingival portion of an acute trench mouth is one and the same thing as other gingivitis and the acute flareup is due to cultural characteristics and conditions. The acute ulcerative and exudative gingivitis progresses to stomatitis, the condition now recognized as trench mouth.

6. The study and future treatment of gingivitis depends upon an intelligent and close cooperation between the laboratory and the dentist. When this exists with the patient's cooperation, results may be expected in every case. This has a good many "ifs," but the complexity of the problem demands it at the present development of our knowledge.

7. The high points of a study of over 4,000 cases are reported from an etiologic standpoint, and the following conclusions are drawn:

A. 96.4 per cent show the presence of spironema.
71.6 per cent show the presence of E. gingivalis (Gros).
66.6 per cent show a definite bacterial flora.

B. These three groups of organisms alone or in combination are the direct etiologic factors in every case of gingivitis, and all other factors present are contributory and subservient.

C. The spironema and E. gingivalis and bacteria act mechanically, finding an ideal culture bed in the gingival sulci in which to set up a gingivitis. The toxic factor of each is probably secondary but equally important.

D. The spironema and E. gingivalis reside probably only in the gingival sulci while the bacteria penetrate the gingivae, lodging in the deeper structures where they act as potent foci of infection.

E. There is evidence that the so-called symbiotic relationship of Vincent's spirillum and B. fusiformis does not hold, but in reality these are cycles or phases of a higher form of leptothrix.

F. It is suggested that the term Vincent's spirillum is misleading and should be discarded as well as the entity Vincent's angina.

G. Until further light is thrown upon the spirilliform organisms of the mouth, we believe that all of these organisms are one and the same genus and fall best according to the manual of *Determinative Bacteriology* under order VI spirochetales, Family 1 spirochetaceae, Genus IV spironema,* 12. Spironema vincentii (Blanchard). For the time being we think that any association, as a symbiosis or as a necessity for diagnosis, with any fusiform bacilli may be disregarded. We further believe, under Genus V treponema of the same family spirochaetaceae, that 3. Treponema microdenteum (Noguchi), 4. Treponema macrodenteum (Noguchi), and 5. Treponema mucosum (Noguchi) are one and the same as spironema with cultural variations.

H. The disease known as trench mouth is nothing more than a flare-up having its origin in a gingivitis and as such should be spoken of as acute ulcerative gingivitis and stomatitis due to spironema as a part of the general problem of gingivitis.

I. A method of study from the laboratory standpoint is offered with a standardization of reports.

J. Gingivitis is not due to a single specific factor but to a combination of infections which must be studied in every mouth and evaluated for that individual.

K. We believe that the evidence bacteriologically, pathologically, and clinically is sufficient to show definitely the relationship between the organisms encountered and the clinical reactions and to state that all forms of gingivitis have a common etiology with degrees of reaction.

L. We believe that the vast majority of cases start insidiously in young life and continue slowly but progressively, terminating surely and eventually in marked and destructive changes in the middle and later decades.

8. From an intensive study of twelve cases with daily examinations, the constant character of the gingival bacterial flora was striking.

9. The H-ion concentration of gingival exudates would seem to be influenced

*Where the generic name spironema appears in this paper it should be substituted by Borrelia. The generic name spironema used in the first edition of the *Manual of Determinative Bacteriology* is invalid because of its prior use for other organisms and in the second edition it is changed to Borrelia.

by the gas-forming acid organisms of the flora and to maintain in part an acid reaction, which is greater than the saliva of the mouth.

10. The gingivae, the seat of gingivitis, offer the largest and most important single portal of entry for microorganisms and as such are the most potential sources of foci of infection in the body.

11. Organisms picked up by the circulation from the gingival foci are responsible for many distant manifestations and metastatic foci.

12. This latter statement makes a direct connection between this source of infection and such diseases as arthritis, rheumatism, endocarditis, myocarditis, nephritis, anemia, and several more.

13. The treatment depends upon a careful consideration of all contributory factors present in a given case and their complete correction. With this, local chemotherapeutic administration in proper amounts, concentration, time, method, and control carryon, the problem of gingivitis difficult and complex may be solved.

14. From all these different angles it is finally concluded that gingivitis is an entity only in so far as it involves the gums, it is due to several factors, primarily infection with spironema, E. gingivalis, and the bacterial group playing the important rôle, and all other conditions are secondary and contributory. Gingivitis is a chronic disease of long duration in the vast majority of cases, starting mildly but ending in necrosis and destruction. Gingivitis is subject to acute flareups as a part of the general disease. Gingivitis will subside and can be absolutely kept under control by appropriate therapeutic measures intelligently applied.

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LABORATORY METHODS

THE CULTIVATION OF TUBERCLE BACILLI. AN IMPROVED METHOD FOR ISOLATION FROM TUBERCULOUS MATERIALS*

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SINCE the introduction in 1915 of Petroff's[1] method for the primary isolation of tubercle bacilli from tuberculous materials, there have been few advances or improvements in methods for isolating tubercle bacilli, and this method has been considered the best and most efficient by the majority of laboratory workers as well as by clinical pathologists. More recently Löwenstein[2] suggested the use of acids as reagents for treating the tuberculous material before planting on mediums for isolating the tubercle bacilli. The advantages or disadvantages of the acid methods over the sodium hydroxide method have not been thoroughly investigated. The clinical pathologist who wishes to isolate tubercle bacilli from tuberculous materials either for diagnostic or experimental purposes would prefer a method yielding pure cultures in as short a time as possible and with the least expenditure of labor and time for performing the manipulations required. The timesaving factor plays an important rôle in the selection of methods of diagnosis especially and particularly for the isolation of tubercle bacilli, since these organisms grow only slowly at best. For this purpose and as a part of the program of the Research Committee of the American Society of Clinical Pathologists a critical study was made of the relative efficiency of the alkali (Petroff's) and acid (Löwenstein's) methods as well as of several other methods, considering especially the efficiency of the reagents and the suitability of the mediums.

A study of the methods for the isolation of tubercle bacilli from tuberculous materials can conveniently be divided into two parts; namely, one dealing with the efficiency of the reagents in killing off contaminating organisms and the other the efficiency of the medium for the growth of tubercle bacilli. Although these studies were concerned primarily with isolation from positive sputums, the results apply equally well to other tuberculous materials, such as urine, feces, and tuberculous tissues.

I. AN INVESTIGATION OF THE REAGENT USED FOR EXCLUDING CONTAMINATING ORGANISMS

Among the various reagents used in isolating tubercle bacilli, antiformin[3] (Brown and Smith, Paterson, Griffith), formalin (Spengler), and sodium hydroxide (Petroff[4]) have played prominent parts, but the latter was found from our previous studies to be far superior to the other two methods.[5] Likewise glycerine has been suggested as a means of destroying the contaminators,

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but this was also found to be of no service in our laboratory.[6] More recently Dold[7] suggested the use of urea as an efficient agent for destroying contaminators, and Löwenstein[8] advocated the use of sulphuric, hydrochloric and other acids for the same purpose.

In studying germicides for destroying the contaminating organisms, two factors must be given consideration. First, the efficiency of the reagent as a germicide for the contaminators, and second, the toxicity of this reagent for the tubercle bacillus, the organism to be isolated. The first of these factors can be satisfactorily studied by mixing sputum with a definite quantity of the reagent and seeding the final product of such treatment on a standard medium of known efficiency, such as Petroff's gentian violet egg medium, and then determining the number of contaminations resulting in the seeded culture tubes after incubation. The second factor can be determined by taking a definite weighed quantity of tubercle bacilli and, after making a fine suspension in normal saline solution, mixing a definite portion with a definite volume of the reagent and then seeding the final washed or diluted product on to a standard medium and noting the growth of the tubercle bacilli. By the use of these methods we studied the relative value of different reagents for the isolation of tubercle bacilli and have concluded from these studies:

1. *Urea and Sodium Carbonate.*—Urea possesses an excellent solvent action while sodium carbonate exerts a precipitating effect upon the mucus-like materials in sputum and tissues. By means of these reagents tubercle bacilli can be isolated only in a few cases, because they permit frequent contamination of the culture mediums, even when the sputum or other tuberculous material is treated with a high concentration of the reagents. Urea and sodium carbonate are, therefore, unsuited as reagents for destroying the contaminators for the purpose of isolating tubercle bacilli from contaminated sources.

2. *Ammonium Hydroxide* is an efficient germicide and permits the growth of only a small percentage of contaminators even when sputum is treated with a low concentration of the reagent. This reagent, cannot, however, be employed for isolating tubercle bacilli as these bacilli are also very susceptible to its toxic action, and the same low concentrations that permit the growth of contaminators are also injurious to tubercle bacilli.

3. *Ammonium Carbonate* is an inefficient germicide for contaminating organisms and yields too frequent contamination even when sputums are treated with a high concentration of the reagent. At the same time this reagent is too toxic for tubercle bacilli, and is entirely unsuited for the isolation of tubercle bacilli from contaminated sources.

4. *Sodium Hydroxide*, the reagent recommended by Petroff is an efficient germicide to contaminating organisms and is also of low toxicity to tubercle bacilli. Our results using Petroff's medium gave an efficiency of about 55 per cent isolation, using all growths even of small amount as a criterion, and about 16 per cent contamination with positive microscopic sputums, seeding about 3 tubes from each sputum. These figures compare favorably with most previous investigators.

5. *Sulphuric Acid and Hydrochloric Acid.*—The acid reagents recommended by Löwenstein for isolating tubercle bacilli are efficient germicides toward contaminating organisms and possess little toxicity toward tubercle bacilli in suitable concentrations. They also possess the advantage of permitting an earlier appearance of colonies as compared with cultures when sodium hydroxide has been used. In an experiment with four positive sputums, using the sulphuric acid reagent and on the same sputums the sodium hydroxide method, all seeded on the same medium, growth occurred several weeks earlier with the sulphuric acid reagent than it did after the use of sodium hydroxide. The percentage of positive isolations were also greater with sulphuric or hydrochloric acid (about 65 per cent and 10 per cent contaminations) when the proper culture medium was used. The superiority of sulphuric and hydrochloric acid over sodium hydroxide is probably accounted for by the fact that in the sodium hydroxide method, the heavy slimy fluid produced by mixture of sodium hydroxide and sputum prevents efficient sedimentation of the tubercle bacilli during centrifugation, while with sulphuric or hydrochloric acid, the sputum reagent mixture produces a flocculent precipitate upon dilution, which materially assists in sedimentation of the bacilli during centrifugation. Sulphuric acid proved slightly superior to hydrochloric acid because the former has a wider range of useful concentration than the latter.

In summarizing the part of the investigation concerning the value of the reagents tested, it is concluded that urea and sodium carbonate are unsatisfactory reagents for the isolation of tubercle bacilli, because frequent contamination occurs after their use, they being inefficient germicides for contaminating organisms in a concentration and time interval tolerated by tubercle bacilli without appreciable injury. Ammonium hydroxide proved too toxic toward tubercle bacilli in concentration and time interval suitable for destroying the contaminators. Ammonium carbonate possesses the same disadvantages. Sulphuric acid, hydrochloric acid, and sodium hydroxide are all suitable in that they destroy contaminators within a time interval of exposure and concentration in which they are innocuous to tubercle bacilli in tuberculous materials. Of these three reagents an equal volume of 6 per cent sulphuric acid and thirty minutes incubation (37° C.) proved superior, with 3 per cent hydrochloric acid in equal volume for from fifteen to thirty minutes incubation (37° C.) next in value, and an equal volume of 2 per cent sodium hydroxide for from fifteen to thirty minutes least efficient in yielding positive cultures of tubercle bacilli as well as in destroying contaminators.

II. THE VALUE OF DIFFERENT CULTURE MEDIUMS

To prevent further the growth of such contaminating organisms as were not destroyed by the sodium hydroxide reagent, Petroff incorporated gentian violet as a bacteriostatic agent in Dorset's egg medium. The gentian violet possesses an added advantage of making the detection of colonies of tubercle bacilli on the medium easier. Prior to the World War of 1914-1918, G. Grübler and Company, of Germany, was the sole distributor of dyes in this country. After the war the dye manufacturers and biologists of the United States organized a "Commission on the Standardization of Biologic Stains"

in order to stabilize and standardize the dyes used in America. Accordingly on the recommendation of the commission, dye manufacturers are now placing on the market "crystal violet" or "methyl violet" as a substitute for Grübler's gentian violet.

A number of samples of the dyes manufactured by the leading dye manufacturers in this country and kindly furnished to us by Dr. H. J. Conn, Chairman of the Commission on Standardization of Biologic Stains, have been tested for their action on tubercle bacilli. The samples of the dye tested included the following: (1) crystal violet from the National Aniline and Chemical Company, sold as gentian violet; (2) crystal violet from the Coleman and Bell Company, sold as gentian violet; (3) crystal violet from the Empire Biochemical Company, sold as gentian violet; (4) crystal violet from the Hartman-Leddon Company, sold as gentian violet; (5) methyl violet from Coleman and Bell Company also sold by them as gentian violet and (6) crystal violet from the Coleman and Bell Company, sold as crystal violet. The study of the relative value of these dyes indicates that when they are present in high concentrations (0.01 per cent or over) in mediums they inhibit the

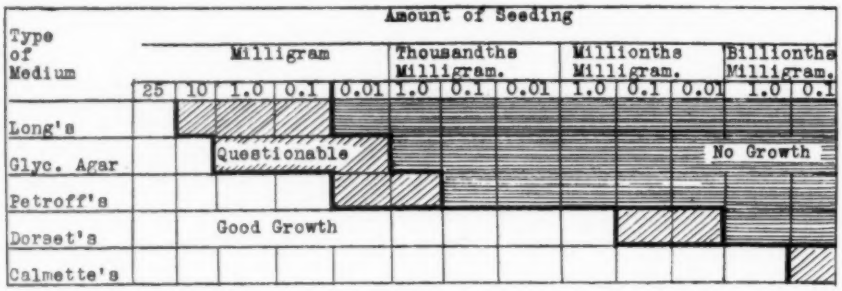

Fig. 1.—Growth chart for a strain of virulent human tubercle bacilli (Gluckson) when seeded in dilute suspension on different media.

growth of tubercle bacilli, while in lower concentrations they exert no demontrable injurious effect. Any of these dyes can be used in egg mediums in the same concentration (1:10,000) as originally recommended by Petroff for Grübler's gentian violet.

In a previous study on the relation of food accessory substances (vitamins) to bacterial growth[9] a suspension method for seeding tubercle bacilli in graded dilutions was used in order to compare the efficiency of different mediums for the growth of tubercle bacilli. This same method was used in the following investigation for the purpose of comparing the efficiency of different mediums for the growth of tubercle bacilli. A number of interesting findings have resulted from this study. Fig. 1 depicts at a glance the relative efficiency of the different mediums tried when tubercle bacilli are seeded on them in graded amounts from dilute suspensions. It is evident from the figure that growth of virulent human tubercle bacilli on the nonprotein medium of Long occurs only when the medium is seeded with suspensions containing 25 mg. or more of bacilli per cubic centimeter. When the concentration of the suspension is from 0.1 mg. to 10 mg. per cubic centimeter, there may or may not result growth, while when the inoculum contains only 0.01

mg. or less of bacilli per cubic centimeter, no growth occurs in any of the tubes seeded. On 5 per cent glycerin nutrient agar medium, growth takes place when the concentration is 10 mg. or more of bacilli per cubic centimeter, while a concentration of from 0.01 mg. to 1 mg. of bacilli per cubic centimeter gives uncertain growth. With 0.001 mg. or less bacilli per cubic centimeter, seeding fails to give colonies on the glycerin agar medium. On Petroff's gentian violet egg medium, growth occurs when the suspension contains 0.1 mg. or more of bacilli per cubic centimeter, while between 0.001 and 0.01 mg. per c.c. uncertain growth results, and with 0.0001 mg. or less per cubic centimeter the seedings entirely fail to grow. With Dorset's and Calmette's medium, these points of extinction have not yet been fully determined, but it seems that with Dorset's medium, growth always takes place at the concentration of 0.000,001 mg. or more, while below 0.000,000,01 mg. growth does not take place. On Calmette's medium growth occurs down to 0.000,000,001 milligram with certainty. From this experiment it is also to be noted that on Calmette's potato cylinder medium, tubercle bacilli grow more luxuriantly and develop visible colonies earlier than on the other mediums tested. This was especially so with the high dilutions of the bacilli. In an earlier study[10] it was computed that 1 milligram of tubercle bacilli taken from a culture contained between 5.01 and 7.56 billion tubercle bacilli, or, theoretically speaking, if grinding was thorough, a suspension of 0.000,000,000,1 mg. of bacilli per c.c. should contain only a few bacilli. Since only 3 or 4 drops (about 0.3 c.c.) of suspension were used for seeding the culture tubes, this dilution would give only one or two colonies or none at all. Although definite statements are not justified from our preliminary experiments, it is interesting that in this concentration a few colonies of tubercle bacilli developed on the potato medium. The guinea pig inoculation method for determining the presence of tubercle bacilli is considered the most sensitive available for this purpose at present and has proved to be one of the most important means for the diagnosis of doubtful cases of tuberculosis. The use of the guinea pig for this purpose has a number of disadvantages in not being easily accessible, being more expensive, and in requiring greater facilities than the culture method. If it were possible to use the culture method with equal efficiency to the animal test, it would make possible not only a test susceptible of repeated examinations but would also prove to be time and labor saving. This is now being investigated in our laboratory.

Although the potato cylinder medium proved to be the most satisfactory medium for the growth of tubercle bacilli, its use unmodified for the primary isolation of tubercle bacilli was impaired by the fact that contaminators also thrived on it. The contaminations diminished the percentage of isolation of tubercle bacilli from sputum as compared with the results on Petroff's gentian violet egg medium. Löwenstein attempted to use mercuric chloride in the potato medium in order to prevent the growth of contaminating organisms, but we have found this reagent highly toxic to tubercle bacilli as well as to other organisms. To remedy this defect of the potato cylinder medium, crystal violet was introduced; thus we have succeeded in preparing a medium apparently exceeding the value of the original potato cylinder medium in pro-

ducing an acceleration of the growth of the tubercle bacilli. This stimulating effect of the small amount of crystal violet in the medium upon the growth of the tubercle bacilli may be analogous to the stimulation in the growth of plants occasioned by the presence in their nutrient mediums of a minute amount of the salts of heavy metals, which if present in a larger concentration exert a deleterious effect. In an earlier report it was also pointed out that a small amount of carbon dioxide in the surrounding atmosphere accelerated the growth of tubercle bacilli while higher concentrations exerted a harmful influence. Thus, it is conceivable that crystal violet in low concentration accelerates the growth of tubercle bacilli, while in higher concentrations it may retard their growth.

The concentration of crystal violet still suitable for use in the potato cylinder medium for the isolation of tubercle bacilli is 0.01 per cent or less. The

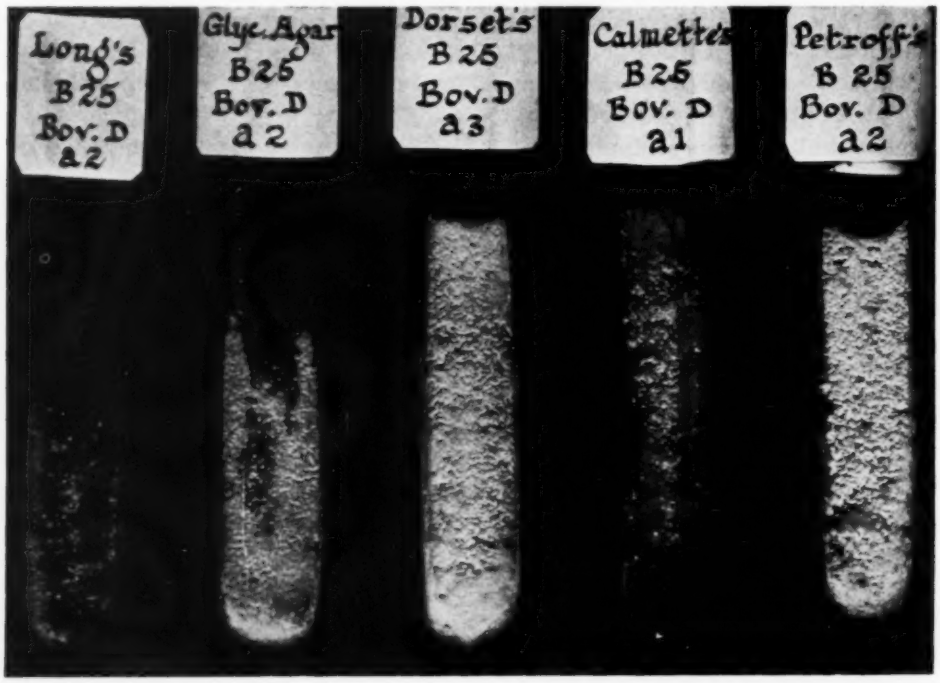

Fig. 2.—The growth of tubercle bacilli (bovine) on Long's nonprotein medium, five per cent glycerol agar, Dorset's plain egg medium, potato cylinder nutrient broth and Petroff's gentian violet egg medium 5 weeks after seeding with a few drops of suspension containing 25 milligrams in a cubic centimeter and incubating at 37° C. Human bacilli grow about like bovine on these mediums. Note less profuse growth on Long's and potato medium with heavy seeding.

most favorable concentration in our experiments was found to be 0.003 per cent or about a 1:75,000 dilution. The crystal violet potato cylinder medium as well as the plain potato cylinder medium proved superior to Petroff's and other mediums tested, in so far as the first appearance of colony formation was concerned after seeding with tubercle bacilli. This combined with the use of the 6 per cent sulphuric acid reagent resulted in a saving of several weeks time in many instances of isolation of tubercle bacilli from contaminated sources.

Regarding the substances present in the potato medium favorable to the growth of tubercle bacilli little is known, and in elucidation of this it may be noted that Nocard and Roux's glycerin nutrient agar medium contains sufficient glycerin and bouillon for the growth of tubercle bacilli, yet it fails to support growth consistently when the number of bacilli are less than 10 milligrams per cubic centimeter. Löwenstein found that potato cubes dipped in saline solution alone may be used for the cultivation of tubercle bacilli. From all the evidence available it would appear that the stimulating substance for the growth of the bacilli is present in the potato rather than in the bouillon or glycerin. The studies of Thjötta and Avery[11] have shown that there is present in potato two distinct and separable substances which accel-

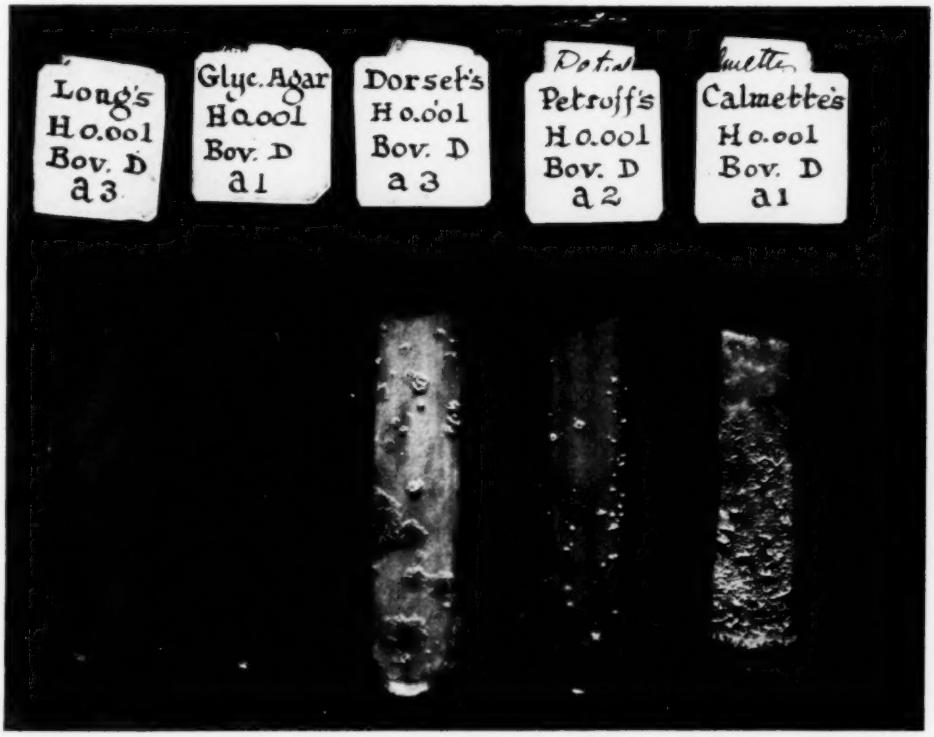

Fig. 3.—The growth of tubercle bacilli (bovine) on Long's, glycerol agar, Dorset's, Petroff's and potato mediums 6 weeks after seeding with a suspension containing 0.001 milligram in a cubic centimeter and incubating at 37° C. Note absence of growth on Long's and glycerol agar mediums with this seeding and more profuse growth on the potato medium than on Dorset's medium, and least growth on Petroff's medium.

erate the growth of hemophilic bacteria, one a heat stable so-called X substance and another a heat labile vitamin-like substance. In a recent work[12] it was found that tubercle bacilli thinly seeded in small amount on synthetic nonprotein medium fail to grow, while in the presence of substances rich in vitamine B there is a marked acceleration of growth. This might incline one to believe that the active principle in potato suitable for the growth of tubercle bacilli may possibly be a vitamine B-like substance alone or vitamine B-like as well as the X substance of Thjötta and Avery. Potato starch was

tested as a substitute for raw potato, but it was found that although the potato starch promotes the growth of the bacilli when the medium is heavily seeded, with small amounts of seeding no stimulating effect was discernible. The growth-promoting principle appears, therefore, to reside mainly in the raw potato and this is being submitted to further study.

AN IMPROVED METHOD FOR THE PRIMARY ISOLATION OF TUBERCLE BACILLI

As a result of a study of various reagents and mediums the following method is proposed as being an advance over methods previously used for this

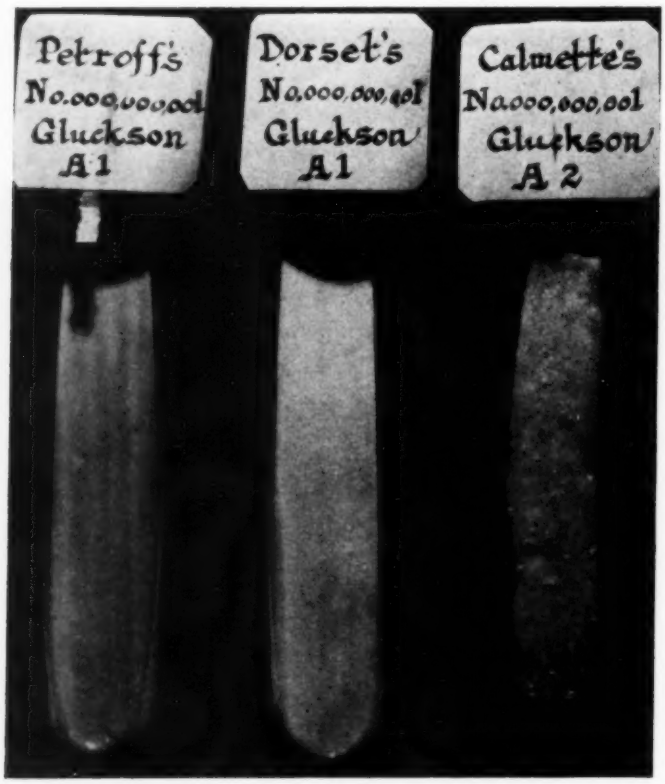

Fig. 4.—The growth of tubercle bacilli (virulent human) on Petroff's, Dorset's and potato mediums 5 weeks after seeding with a suspension containing 0.000,000,001 milligram in a cubic centimeter and incubating at 37° C. Note small amount of growth on potato medium and its absence on the other two mediums.

purpose. The method is suitable for use in isolating pure cultures of tubercle bacilli from any contaminated sources, such as urine, feces, tissues, and pathologic fluids as well as sputums.

Preparation of Reagents.—To prepare 6 per cent sulphuric acid solution, take 3.4 c.c. of concentrated sulphuric acid (specific gravity 1.84 H_2SO_4 content 95-96 per cent) and cautiously pour into distilled water to a final volume of 100 cubic centimeters. To prepare 3 per cent hydrochloric acid, 7.1 c.c.

of concentrated hydrochloric acid (specific gravity 1.18; HCl 35-36 per cent) are diluted to 100 c.c. with distilled water.

Both of these acids have proved suitable.

Preparation of the Medium.—Large clean potatoes free from surface defects are cut into cylinders about 3 inches in length and ⅝ inch in diameter, using for this purpose a cork borer. They are then halved longitudinally. As soon as they are cut, these potato cylinders are soaked in 1 per cent sodium carbonate solution containing crystal violet (the stain added just prior to use since prolonged contact with the sodium carbonate leads to decolorization) in concentration of 1:75,000 or 0.003 per cent. After soaking from one to two hours, they are gently wiped with a clean towel to free them from excess of fluid and are then introduced into a sterile culture tube (6 by ¾ inch in size) containing 1½ cubic centimeters of 5 per cent glycerin bouillon, cotton plugged, and sterilized in an autoclave at 15 pounds pressure for at least thirty minutes.

Treatment of Sputum or Tuberculous Material, and Seeding.—Sputum or tuberculous material is first thoroughly beaten up to a homogeneous pulp and 1 c.c. of the resultant fluid is introduced into a sterile centrifuge tube of 15 c.c. capacity. One cubic centimeter of 6 per cent sulphuric acid or 1 c.c. of 3 per cent hydrochloric acid is added, and the contents are thoroughly mixed together. Too much emphasis cannot be placed on the thoroughness of mixing, since the number of contaminations depend greatly upon this. The tube is stoppered with a sterile cork and incubated at 37° C. for thirty minutes with occasional shaking. At the end of thirty minutes the contents are diluted with about 10 c.c. of sterile 0.9 per cent saline solution, well mixed and centrifuged. The supernatant liquid is decanted, and the remaining fluid measuring from about 1 to 2 c.c. is well mixed by means of a sterile pipette and is seeded on the surface of the crystal violet potato cylinder medium. After being cotton-plugged and capped with tin foil or with light paraffin impregnation of the cotton, the culture tubes are kept in an incubator at 37° C. Within from two to five weeks a luxuriant elevated growth of tubercle bacilli becomes visible, especially above the surface of the 5 per cent glycerin bouillon.

A NOTE ON KEEPING CULTURE TUBES

Often when spores of molds are abundant in the air, these germinate on the surface of the cotton plugs of the culture tubes and finally penetrate down through the stopper into the medium, thus spoiling cultures of tubercle bacilli. In laboratories where large numbers of cultures are being kept over a long time, this loss may become considerable. Frequently during the course of isolating primary cultures or perpetuating cultures of tubercle bacilli, the prevention of mold growth plays an important rôle in the success of these endeavors. Any method used to prevent the growth of molds must also consider and make provision for retaining the optimum conditions for the growth of the tubercle bacilli. The following several methods were studied because some had been recommended as superior to the usual method of using a plain paraffined cotton plug or a tin foil cap: I. Plain nonabsorbent cotton plug

(a) full-dipped in melted paraffin; (b) half-dipped in paraffin; (c) untreated with paraffin. II. Nonabsorbent cotton plug previously treated with 1:500 mercuric chloride solution (a) full-dipped in melted paraffin; (b) half-dipped in paraffin; (c) untreated with paraffin. III. Nonabsorbent cotton plug previously treated with 1 per cent sodium salicylate (a) full-dipped in melted paraffin; (b) half-dipped in melted paraffin; (c) untreated with paraffin. IV. Effect of naphthalene vapor—untreated with paraffin.

In all the cases above except IV, The Study of the Effect of Naphthalene Vapor, eight tubes for each subdivision (namely, four tubes for 5 per cent glycerin agar nutrient medium and four tubes for Petroff's gentian violet medium) were seeded with suspensions of tubercle bacilli. One-half of these tubes were kept in a moist can while the other half were kept in a dry can, and the number of contaminations by molds and the rate of growth of tubercle bacilli were noted. At the end of the fourteenth week of observation it was concluded that the use of dry cans for storing culture tubes should be recommended as compared to the use of moist cans, since the growth of molds is more abundant and common in the latter at incubator temperature. If culture tubes are to be kept for longer than four weeks in the incubator, they should be placed in dry metal cans provided with a tight (not air-tight) cover in order to prevent excessive drying of the medium.

In spite of the recommendation by Soparkar[13] that the cotton be soaked in mercuric chloride or sodium salicylate, it was found that culture tubes plugged with a plain cotton plug gave superior growth to those provided with cotton plug which has been previously treated with mercuric chloride (1:500) or sodium salicylate (1 per cent). The toxic effect of mercuric chloride was very evident, being less so but still perceptible with sodium salicylate. The use of naphthalene as an antimold measure also proved unsatisfactory, on account of its deleterious action on the growth of the tubercle bacilli.

As a result of a comparison of tests with full-dipped, half-dipped in melted paraffin and untreated cotton plugs, it was found that the cotton plugs half-dipped in paraffin are the most desirable. Cotton plugs not treated with melted paraffin also gave good results but tended to result in early drying up of the culture medium as well as in a greater likelihood of contamination with molds. Cotton plugs heavily soaked with paraffin cannot be recommended because this not only results in an inferior growth of tubercle bacilli but frequently may result in complete absence of growth, due to the inaccessibility of atmospheric air.

SUMMARY

Of the various reagents used for the primary isolation of tubercle bacilli from contaminated tuberculous materials, sulphuric acid and hydrochloric acid were found superior to any other reagents tried, including sodium hydroxide, for destroying the undesirable contaminators present.

Standard crystal violet or methyl violet of the American dyes was found serviceable in replacing the prewar Grübler's gentian violet, and in the same concentration, for preparing Petroff's gentian violet egg medium.

Crystal violet potato cylinder medium is superior to gentian violet egg medium, Dorsett's egg medium, and other mediums for the cultivation and isolation of tubercle bacilli.

A new method is described for the isolation of tubercle bacilli from contaminated tuberculous sources, incorporating the use of sulphuric acid (or hydrochloric acid) as a reagent for preliminary treatment of the materials and crystal violet potato medium as a nutrient medium for their growth.

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DISCUSSION

Dr. Robert A. Keilty.—One of my first loves was tuberculosis. In 1915 I was able to publish one of the first papers on Petroff's method so I am particularly interested in this subject. The potato medium appears to give a more luxuriant growth than is obtained on Petroff's medium. The cultivation procedure is divided into two parts: the destruction of contaminators by acid or alkali and the continuation of retardation by the introduction of some substance into the culture medium. In spite of all the work that has been done on dyes, it is almost impossible to tell what you have as they come to you from the manufacturer. The variation in these dyes is anywhere from 35 to 38 per cent of dye activity. I believe that there has been only one of the aniline dyes actually standardized. That is very important when it comes to knowing the amount of material put into the culture medium. In this method the dyes exert an individual specific chemostatic action.

Dr. C. E. Royce.—What is the method of sterilization of the medium?

Dr. Corper.—The method is as follows: Take an ordinary potato, peel, cut to cylinder and divide it in half, wash, and dip in 0.003 per cent gentian violet in 1 per cent sodium

carbonate solution for two hours. Then autoclave at ordinary high pressure for from about thirty minutes to one hour, after tubing with 1.5 c.c. glycerol broth.

Dr. Frank W. Hartman.—Did you try the effect of oxygen and carbon dioxide tension?

Dr. Corper.—The effect of carbon dioxide and oxygen upon the growth of tubercle bacilli has been extensively studied in our laboratory, and this was considered in these cultivation studies. Suffice to say briefly that the tubercle bacilli grow best in atmospheric air on most culture mediums, although with lower concentrations of oxygen a small amount of carbon dioxide exerts a beneficial influence, while higher amounts of carbon dioxide may be inhibitory. On the potato medium atmospheric air supplies ample oxygen, and the bacilli grow better than in other gas mixtures.

Dr. George Ives.—Will Dr. Corper make a few remarks as to the use of the guinea pig in diagnosis; the time it takes tuberculosis to kill a guinea pig, etc. We often want a quick diagnosis. This subject will be interesting.

Dr. Corper (closing).—It must be admitted that any method requiring a wait of a couple of months to make a diagnosis is fraught with difficulty. This is the objection to the use of the guinea pig. I told you before that the guinea pig is an extremely susceptible animal to inoculation tuberculosis and that it requires only from 10 to 100 bacilli from the ordinary sputum to infect this animal. The question of the importance of the route of infection has led to much discussion. You have heard of the many recommendations and preferences. Some prefer injecting intravenously, others into the mammary gland, some the intraperitoneal route, etc. In our own laboratory we have exhaustively investigated the importance of the routes of infection in the guinea pig as well as the effect of various reagents upon the infection in this animal, bearing in mind its importance for the clinical pathologist. The results have been presented to you at previous meetings of this Society. The guinea pig and monkey are extremely susceptible to inoculation infection with tubercle bacilli as few as 10 to 100 bacilli being sufficient to produce a generalized disease in from two to three months. Therefore, whether you introduce the bacilli intravenously, subcutaneously, or intraperitoneally makes little difference, because of the extreme susceptibility of these animals. Our choice has been subcutaneous inoculation for the animals need not be sacrificed and there is a visible index of infection. The local gland begins to enlarge, and upon incising at the appropriate time, a small amount of pus can be obtained for preliminary diagnostic examination. If acid-fast bacilli are found, a tentative diagnosis can be made, and at a late date (after a few weeks more) the guinea pig can be sacrificed and the diagnosis verified by finding a generalized tuberculosis in this animal. The enlargement of the local gland depends largely upon the number of bacilli that were present in the inoculated material; with few bacilli it may require a month or two, while when large numbers are present, the local gland will become visibly enlarged and contain pus in a few weeks.

VASCULAR INJECTION IN PATHOLOGY*

By Ernest Scott, M.D., and Robert A. Moore, B.A., Columbus, Ohio

IN THE past a large number of methods have been developed for the study of the hollow organs of the body, more especially the arteries, capillaries, veins, and ducts of the glands. In connection with studies on the vascular changes in nephritis being carried on in this laboratory, it seemed advisable to correlate these various methods and to deduce from them some general principles which may be followed in any type of injection. These principles and a standardization of technic are of the utmost importance in pathologic studies. In anatomy it is desirable, at least in some cases, that the injection be incomplete since any attempt to unravel the total circulation is impossible, while if only a part is shown at one time, the relationships can be readily seen and followed. In pathology, if a portion of an organ is not injected, the question immediately arises, is this noninjection due to some pathologic change or is it a result of faulty technic?

With this viewpoint in mind, a standard technic becomes paramount; yet any attempt at standardization is of little value if all the various methods of injection cannot be controlled, so that the one standardization applies to all. It is this problem which we have attempted to solve in order that any one pathologic condition may be studied by any or all of the methods available and different views secured of the same process.

TYPES OF INJECTION

The various types of injection and injection masses may be conveniently divided as follows:

1. Fluid masses to be injected at room temperature.
2. Fluid masses which are injected in warm, fluid state and are then allowed to set in the vessels.
3. Masses which are injected as a fluid, but which set in the vessel and the tissues later removed by erosion.
4. Masses which are opaque to the x-ray.
5. Specific stains.

The cold masses are the easiest to use but they do not give the beautiful pictures which can be secured with other methods. As such an injection mass Lee-Brown[1] recommends 2 per cent soluble Berlin blue in water as giving excellent results. Prussian blue is also good and may be prepared by adding equimolecular proportions of chemicals and diluting so that the result of the mixture (Prussian blue) is in a concentration of 2 per cent. Müller[2] used Berlin blue in 1 per cent glue. Other workers have used injections of two fluids which when they meet in the vessels cause the formation

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of a precipitate. Bowman[3] thus used a method devised by Krause consisting of potassium dichromate and lead acetate injected successively and giving a precipitate of lead chromate. Bird and Moise[4] injected a solution containing iron ammonium citrate and potassium ferrocyanide and fixed small pieces in acid formalin. The acid in the fixative caused the precipitation of Prussian blue wherever the solution had injected. By this method they were able to inject the tubular system of the kidney. Kerr and Meffier[5] have used India ink in concentration of 50 per cent in distilled water for injection of the capillaries of the heart valves. We have used 50 per cent India ink in distilled water to inject the bursa about the bundle of His in the beef heart, and secured very excellent specimens. The technic of this injection is given by Kaufmann.[6] Milk injections and subsequent staining of the fat globules have been used.[7]

Warm injection masses, particularly gelatin carmine, give results which are not excelled by any other one method. Gelatin is the usual base of the mass, although starch and other substances have been used. This type of injection may be divided into those in which the gelatin is stained before injection and those in which the staining of the injection is accomplished in sections prepared from the organ injected.

Gelatin masses are usually colored with Berlin blue or carmine. The former is not difficult of preparation.[7] It seems desirable, however, that arterial injections should be in red; the usual method of the preparation of carmine injection masses as given by Mallory and Wright[7] is rather capricious and of little value when consistent and standard results are desired. MacCallum[8] has recently published a method for the preparation of gelatin carmine in which the excess ammonia was removed from the carmine solution by boiling, before adding to the gelatin. Bensley[9] has prepared carmine in a colloidal state and added this to the gelatin. However, in attempting to secure a satisfactory gelatin carmine by a short method, it occurred to one of us to use the H electrode to adjust the reaction.[10] By this method we have uniformly secured excellent masses and with very little difficulty. Robinson[11] has secured excellent preparations of the circulation in the spleen by the injection of plain gelatin and later staining with iron hematoxylin.

In any of the gelatin masses it is sometimes of advantage to add about 5 per cent of potassium iodide to reduce the gelatin point in order that manipulation need not be carried out at so high a temperature.

The celloidin corrosion methods are particularly applicable to the demonstration of gross alterations in the larger vessels, but by careful attention to detail good preparations for microscopic study can also be secured. Schiefferdecker[12] first proposed the use of celloidin as the injection mass. He dissolved the celloidin in ether and used asphalt as the coloring matter. This method has the disadvantage that the specimens shrink considerably. Hochstetter[13] modified the technic by using kaolin to prevent shrinkage. The method as used today was developed by Krassuskaya,[14] who dissolved the celloidin in acetone and used camphor to prevent shrinkage. Huber[15] has used this method with excellent results to demonstrate the origin of the arteriolae rectae. Hinman and Morrison[1] have used the method, substituting

used x-ray films for the celloidin in the stronger solutions. Numerous dyes have been used to color the mass, among which should be mentioned cobalt blue, chrome yellow, cinnabar, Victoria blue, Berlin blue, Prussian blue, asphalt, alkanin, and a mixture of crystal violet and brilliant green. The specimens in which Victoria blue is used have the advantage that they may be preserved in a dry condition. Our experience with the celloidin method has been very satisfactory and the formulas which have been used are as follows:

For the pelvis of the kidney:

Acetone	100	c.c.
Celloidin	20	gm.
Camphor	16	gm.

For the larger blood vessels:

Acetone	100	c.c.
Celloidin	10	gm.
Camphor	8	gm.

For the glomeruli:

Acetone	100	c.c.
Celloidin	2.75	gm.
Camphor	2.0	gm.

The exact strength of the latter solution is adjusted to a known viscosity as given later in this paper. All solutions are prepared from pyroxylin, Du Pont. We have found scarlet R. satisfactory as a red stain and methyl green as a bluish green. In place of these dyes, one may use ordinary oil pigments with equally satisfying results. The concentration of scarlet R. should be greater in the thin solution than in the thick; a concentration of 0.3 per cent has been found satisfactory for the thin solution.

After the injection the tissues may be removed by either pepsin 1:3000 in 0.4 per cent HCl or 75 per cent HCl. After from twenty-four to forty-eight hours the softened parenchyma may be removed with a gentle stream of water. For preservation of the specimen the solution recommended by Hinman et al[1] consists of distilled water, 100 c.c., formalin, 2 c.c., and glycerin, 20 c.c. Specimens may also be shown to advantage by mounting in gelatin, according to the technic of Delephine.[17] Such specimens, mounted so that they may be viewed by transmitted light, give striking pictures of the richness of capillary circulation.

Ghoreyet[18] has used the corrosion method to study the circulation of chronic nephritis and the coronary arteries. Instead of celloidin he injected Babbitt metal at 85° C. after irrigation with Adler's salt solution at 85° C. for 90 seconds. His preparations as illustrated give in graphic form the relation of circulation to the severity of chronic nephritis in a most excellent manner.

The first attempts with x-ray opaque materials was with metallic mercury. More recently two types of injection have been developed, one using a cold fluid mass and another using a warm gelatin or lipoidal base. Beck[19] used a suspension of bismuth subnitrate in petrolatum. Katzenstein[20] suspended the bismuth subnitrate in gelatin. Lee-Brown[1] recommends a suspension of finely divided *barium sulphate* in *50 per cent potassium iodide*. We

have used the latter method with good results. In an attempt to secure a finer injection we have prepared a solution of potassium iodide in water and glycerin, but the richness of circulation prevented the securing of any roentgenograms of value. Our impression of this method is that it is very satisfactory for the larger vessels, especially if stereoscopic roentgenograms are made, but that for a detail study, it is of no value.

Specific stains that have been used are silver nitrate and Janus green. The silver nitrate methods are so capricious that they need not be discussed here. Janus green as a specific stain for endothelium has been utilized by Bensley with brilliant results.[21] Recently MacCallum[5] from Bensley's laboratory has published results secured by using a combination of Janus green and gelatin carmine. This method gives the clearest picture of the finer circulation of any that we have used. The Janus green B in aqueous (1:10,000) solution injected into the arterial tree continues on into the veins. After injection the organ is allowed to stand until the dye has been largely changed to a red color, then the Janus green is washed from the vessels by warm saline solution, and the vessels are slowly irrigated with 2.5 per cent ammonium molybdate to fix the blue dye remaining; then gelatin carmine is injected in the usual manner. In this way the endothelium of the arteries and glomeruli are stained a greenish blue while all the vessels, arteries and veins, are filled with the red gelatin. Thus, the unraveling of the circulation into arteries and veins is materially facilitated. This method is only applicable to living tissue.

THE TECHNIC OF INJECTION

The steps in the injection of an organ may be divided conveniently into: first, preparation of the tissue for injection; second, the actual injection, and third, the after treatment and preparation for demonstration.

The preparation of the tissue involves at least two types of manipulation: first, the removal of the blood from the vessels, and second, the securing of a closed vascular system.

For the irrigation of the vessels to remove the blood, Ghoreyet[18] used dropsical fluid. Hinman et al[1] recommended saline. We have used both normal saline and tap water and have not been able to detect any material difference. When one is working with a supravital stain, as Janus green, it is advisable to use warm normal saline, but for all ordinary injections one may simply connect a cannula in the artery or vein to a city water hydrant and allow the water to flow through the vessels until the return flow is clear and the organ a pale gray color. The pressure used is approximately 300 mm. Hg. In the preparation of kidneys for celloidin injections, it has been our policy to use the above technic, with the water flowing at a rate which allows about 150 gallons to flow through the capillary bed in twelve hours.

Clot dissolvers, such as 10 per cent sodium sulphate, have been recommended, but in our experience they are of little value. If within an hour the vascular tree is not free of blood, it probably never will be, regardless of the pressure or solution that is used.

Other workers recommend the use of vasodilators. In the case of animals, the nitrites have been administered before death to secure vasodilatation. We have used this procedure on several rabbits and failed to secure better results. With organs removed after death, Gross[22] recommended the injection of 10 per cent potassium sulphocyanide to release the postmortem rigor of the vessels. Again, this procedure has not yielded better results in our hands.

When the vascular tree has been cleared of all blood, the next concern is to make it a closed system, open only at the arterial and venous ends. Hinman et al[1] make particular mention that no air should be allowed to enter the vessels, and in accordance with this idea, in our early work water was injected and the cut vessels were found by the jet of water springing from them and were ligated. This is a laborious process, and it is difficult to locate all the vessels. To circumvent this, we decided to use air. In the technic the organ is immersed in water, and air under a head of 200 mm. Hg. is forced into the vessels. In this manner the presence of severed vessels is shown by a stream of air bubbles, and in a few moments the ligations can be completed. As yet we have not seen any untoward results from the injection of the air. In other words, we secure just as good results after filling the vessels with air as when precautions were taken to prevent the entrance of air.

With the organ ready for the actual injection, one is next concerned with the injection mass, the types and preparation of which have been discussed in the first part of this paper. It only remains to attempt a standardization which will include all the different types.

It is a well-known fact that the rate of flow of a fluid in a closed tube depends chiefly on two factors: the diameter of the tube and the viscosity of the fluid. Theoretically then one should be able either to reduce the diameter of the tube or to increase the viscosity of the fluid to a point where the fluid will not flow. In dealing with the organs of the body, the size of the tube (capillaries) is fixed for that particular organ, and if one desires to cause a cessation of flow of the fluid through this vessel, the viscosity must be increased. Following this principle, we have carried out experiments to determine the concentration of celloidin in acetone which when injected into the kidney will have a viscosity such that it will fill the glomerular capillaries, but its viscosity is just above the point at which it will flow through the efferent vessels of the glomerulus. The results of these experiments were that a solution with a relative viscosity of 18.5 (compared to water) possessed this property. To confirm the applicability of this principle, we have prepared gelatin and adjusted its viscosity to this point and injected other human kidneys. The results were that the gelatin carmine passed to the efferent vessels but not beyond. (NOTE: Gelatin possesses the property of hysteresis, hence the time elapsing from the melting of the mixture to the measurement of its viscosity must be considered.) The approximate strength of the celloidin mass of such viscosity is 2.75 per cent celloidin and 2 per cent camphor in acetone. Of course this principle does not apply to any mass in which solid particles are suspended, nor does it of

necessity apply to other organs of the same or other species of animals, but it does make possible uniform injections where numerous specimens of the same organ are to be compared and an attempt made to show pathologic change. In using cold fluid masses, glycerin may be added to the mass to increase the viscosity.

The next problem is the pressure to be used and how this pressure is to be applied. This may conveniently be divided into cases in which only a part of the entire length of the vascular tree is to be injected and those cases in which the entire circulation, arteries, capillaries and veins, is to be injected. In either case it will be found convenient to use a tank of compressed gas with an adjustable reducing valve attached, so that any pressure from 50 to 600 mm. of Hg. may be secured and this pressure kept constant for hours. In general the following pressures will be found satisfactory:

Arterial system	600 mm. Hg.
Venous system	250 mm. Hg.
Ductal system	20 to 80 mm. Hg.

The maintaining of these pressures over long periods of time is important. In the case of partial injection, say a celloidin injection up to and including the glomeruli, we have found that for consistent and satisfactory results the pressure should be maintained for twenty-four hours. The organ is immersed in water, and the thin celloidin injected under a constant pressure of 600 mm. of Hg. for six hours. At the end of this time the thin solution is replaced by the 10 per cent solution and the injection continued for eighteen hours longer. We believe that by this technic, with a definite obstruction at the efferent vessel, the celloidin is pushed in all the finer vessels, which it would not enter if the pressure were only maintained for a short time. Further, by immersing in water, the acetone is attracted to the water from the celloidin, thereby precipitating the celloidin. Thus, a little space is left, and as the pressure is still present, more celloidin enters. By this process one secures an accurate cast of the lumen of the blood vessels.

When the injection mass passes through the entire system, a different problem is presented. In this case there is no obstruction to the free flow and if, as usually occurs, a certain pathway through the organ offers less resistance, this part will inject perfectly, and the remainder will inject only in parts. An obstruction could easily be offered by clamping the vein, but the high pressure used in arterial injection would soon rupture the veins and spoil the specimen. In our work we have used intermittent clamping of the vein, thus for a few moments causing an increase of pressure in the vascular tree and forcing the injection mass into the smaller vessels, then releasing and repeating the process several times at short intervals. We believe that this one point will determine, at least in the majority of cases, whether one secures a complete capillary injection or only a useless partial injection.

With the actual injection completed, the question of proper demonstration of the results must be considered. With cold and warm injections the organ is fixed in alcohol or formaldehyde, and sections are prepared in the

usual manner. The warm injections are usually first placed in ice water to set the gelatin, and it has been our policy to place the entire organ in ice cold 10 per cent formaldehyde after injection and leave in the ice box twenty-four hours. With this technic the organ may be cut after twenty-four hours without danger of loss of the injection mass from the smaller vessels. Sections from 10 to 50 microns in thickness may be used or slabs from 1 to 3 mm. may be cut and cleared in aniline oil.

With the corrosion specimens the technic of corrosion and mounting has already been considered. In case one desires to preserve the soft parts and only the larger vessels have been injected, the organs may be cleared by any of the usual methods and the vessels seen in their relationship to the soft parts.

DISCUSSION

The use of injection methods in pathology is twofold: first, for purposes of research, and second, for the clear and concise demonstration to students of vascular changes in an entire organ which cannot be demonstrated by any other method.

Its use in research is already assuming such proportions that we cannot review all the work at this time, but only point out for consideration the organs of the body in which it has been used.

McIndoe and Counsellor[23] have studied the changes in the portal system in atrophic cirrhosis and in the ductal system in cases of long-standing obstructive lesions. They have also investigated the supposed dilatation of the biliary passages in cases of cholecystectomy. In portal cirrhosis they have demonstrated that very little of the portal blood passes through the liver. The question of liver function in these cases must be of importance.

Hinman[27, 28] has investigated extensively the question of hydronephrosis and the related problem of pyelovenous backflow.

Work on nephritis by Gross[16, 29] has already been mentioned. Recently he has devoted an entire book[24] to the question of circulation in the heart valves and apparently has placed the theory of embolic origin of endocarditis on a much firmer foundation.

Ghoreyet[18] has determined the capacity of the coronary circulation in relation to age, and concludes that although the heart increases in size with age, there is not a proportionate increase in the capacity of the coronary arteries.

Terry[25] has injected the circulation in adenomas of the thyroid. He has demonstrated that the veins in these adenomas are very thin-walled and will not withstand even 100 mm. Hg. pressure without rupturing. This fact is offered as a possible explanation of the frequency of hemorrhage in this type of thyroid disease.

Sampson[26] has prepared many injections of fibroid uteri and demonstrated the relationship of vascular supply to these tumors.

SUMMARY

1. The methods available for the study of organs by injection of the vessels or ducts are reviewed.

2. The general principles involved in the technic of securing a complete and satisfactory injection are reviewed.

3. A method of standardization of injection masses by measurement of viscosity is given.

4. A short review of the possibilities of the use of these methods in pathology is given.

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THE RELATIVE DIAGNOSTIC VALUE OF THE LEVINSON TEST AND THE GLUCOSE CONTENT IN CEREBROSPINAL FLUID*

By Alfred S. Giordano, M.D., and Marjorie Ableson,† South Bend, Ind.

IN A PREVIOUS communication[1] it was pointed out that the normal range of the glucose content of the cerebrospinal fluid lies between 60 and 90 mg. Values less than 60 mg. are, in the majority of instances, associated with inflammatory processes in the central nervous system, and, in the absence of pyogenic organisms in such fluids, a tentative diagnosis of tuberculous meningitis can be made.

The validity of this provisional diagnosis, based on a consideration of the glucose content alone, must be established by correlation with other tests and clinical findings. It is generally recognized that the present means of making a definite diagnosis of tuberculous meningitis are limited. The characteristic clinical picture is seldom present in the early stages of the disease; the finding of the organisms in the smear is attended with uncertainty, and cavy inoculation entails too much delay. Hence, it becomes of interest to ascertain the value of any new test which may have diagnostic significance in conditions simulating tuberculous meningitis.

The Levinson test[2] commends itself for investigation because of its simplicity and the rapidity with which results may be read. In this test there is precipitation of proteins as albuminates when the spinal fluid is treated with certain metallic salts and as insoluble salts when it is treated with weak organic acids. The procedure as outlined by Levinson is as follows: One cubic centimeter of spinal fluid is placed in each of two tubes. To Tube 1 is added 1 c.c. of 1 per cent mercuric chloride. To Tube 2 is added 1 c.c. of 3 per cent sulphosalicylic acid. The contents of the tubes are mixed, and the tubes stoppered and allowed to stand for twenty-four hours. The column of precipitate is then measured in millimeters.

Levinson maintains that in cases of purulent meningitis the height of the column of precipitate after the addition of sulphosalicylic acid is three times that following the addition of mercuric chloride, while in cases of tuberculous meningitis the bichloride precipitate is twice that of the acid.

Pons and Fletcher[3] recently reported on a relatively small series of spinal fluids; they were favorably impressed with the value of the Levinson test. They pointed out, however, that the test is not pathognomonic of tuberculous meningitis, since in their experience, in a series of twenty-four spinal fluids from cases of syphilis of the central nervous system, 25 per cent of the positive reactions were false.

Our report is based on the study of ninety-eight spinal fluids from proved

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cases of tuberculous meningitis, syphilis of the central nervous system, purulent meningitis, aseptic meningitis, and varied conditions involving the central nervous system.

METHOD

The method of Folin and Wu, with precipitation of the proteins by the tungstic acid reagents, was used for the quantitative sugar determinations. When the sugar content was less than 25 mg., twice the amounts of filtrate were used, or the dilution of the unknown was made up to 12.5 c.c. instead of 25 c.c., to facilitate the reading. All aseptic spinal fluids with a sugar content of less than 60 mg. were inoculated into guinea pigs to determine the presence or absence of bacilli of tuberculosis. All globulin estimations were made by the Noguchi method using 0.2, 0.5, and 0.1 c.c. The Levinson test was carried out as described and in many instances a duplicate test was made using double concentration of the reagents as suggested by Pons.

RESULTS

Table I shows the results obtained with twenty-three spinal fluids from seventeen proved cases of tuberculous meningitis. The Levinson test was positive in twenty cases (86 per cent); that is, the precipitate with bichloride was twice as high as that with acid. All fluids showed a higher precipitate with mercury, the range being from 1.7:1 to 4.5:1 with an average ratio of 2.7:1. In two of the fluids a precipitate was obtained by using the test solutions in double strength.

The test proved an aid in diagnosis in Case 3 in which the glucose estimation (80 mg.) of the first specimen was misleading. Later specimens

TABLE I

SPINAL FLUID OBSERVATIONS IN TUBERCULOUS MENINGITIS

CASE	HEIGHT IN MM.				RATIO	GLUCOSE, MG.	CHLORIDES, MG.	CELL COUNT	GLOBULIN, GRADE	EXAMINATION OF SMEAR	GUINEA-PIG INOCULATION
	SUL. 3%	$HgCl_2$ 1%	SUL. 6%	$HgCl_2$ 2%							
1	7	15	7	20	1:2.1	37	620	120	4+	Negative	Positive
2	4	11	4	13	1:2.7	37	645	40	2+	Negative	
2						26	655	280	3+	Negative	
2	3	10	4	12	1:3.3	29	665	189	4+	Positive	Positive
3	3	7	4	8	1:2.3	80	580	20	1+	Negative	
3	5	18	5	17	1:3.6	30	625	30	4+	Negative	Positive
4	3	0	2	8	3:0	40	665	50	4+	Negative	
4	3	1	4	7	3:1	51	710	160	2+	Negative	Positive
5	6	7	8	9	1:1.1	54	630	254	2+	Negative	
5	5	15	8	22	1:3	54		240	3+	Negative	
5	7	13	7	15	1:1.8	30	610	260	3+	Positive	Positive
6	1	1.5			1:1.5	28		165	1+	Negative	Positive
7	1	2			1:2	22		98	1+	?	Positive
8	2	9			1:4.5	28	640	430	2+		Positive
9	4	10			1:2.5	5	570	500	4+	Positive	Positive
10	2	7			1:3.5	37	650	278	2+	Negative	Positive
11	3	10			1:3.3	18		192	1+	Negative	Positive
12	5	15			1:3	5		20	1+	Negative	Positive
13	6	14			1:2.3	11		400	2+	Negative	Positive
14	4	9			1:2.2	25		42	2+	Negative	Positive
15	5	10			1:2	0		45	2+	Negative	Positive
16	5	14			1:2.8	26		65	2+	Positive	Positive
17	5	13	7	21	1:2.6	26	585	190	2+	Positive	Positive

TABLE II

SPINAL FLUID OBSERVATIONS IN PURULENT MENINGITIS

CASE	HEIGHT IN MM.				RATIO	GLUCOSE, MG.	CHLORIDES, MG.	CELL COUNT	GLOBULIN, GRADE	ORGANISM FOUND	
	SUL. 3%	$HGCl_2$ 1%	SUL. 6%	$HGCl_2$ 2%						IN SMEAR	ON CULTURE
1	17	16	22	22	1:1	0	700	5000	4+	Pneumococcus	Pneumococcus
2	4	8	4	12	1:2	61	700	1200	4+	Pneumococcus	Pneumococcus
2	4	9	4	14	1:2.2	20	700	1400	4+	Pneumococcus	Pneumococcus
3	9	2			4.5:1	0	548	2000	4+	Pneumococcus	Pneumococcus
4	12	4	13	3	3:1	0	600	1500	4+	Pneumococcus	Pneumococcus
5	0	0			0:0	0		2400	3+	Gram-negative bacillus	Bacillus influenzae
6	2	4	2	10	1:2	68	680	1600	2+	Streptococcus	Hemolytic streptococcus
6	8	6	8	3	1.3:1	5	650	5400	4+	Streptococcus	Hemolytic streptococcus
7	9	0	9	0	9:0	0	620	2000	4+	Streptococcus	Hemolytic streptococcus
8	5	5			1:1	65		700	4+	Streptococcus	Hemolytic streptococcus
9	3	5	4	7	1:1.6	0	640	26000	3+	Diplococcus	Meningococcus
Serum	19	8			2.3:1	42	675	3000	3+	Diplococcus	Meningococcus
Serum	17	10			1.7:1	66	680	2000	2+	Diplococcus	Meningococcus
Serum	14	5			2.8:1	74	715	500	2+	Negative	Sterile
10	8	18	10	20	1:2.2	0	621	11000	4+	Diplococcus	Meningococcus

showed more characteristic glucose values. In Case 5 subsequent tests showed little difference in the height of the precipitates from the first specimen. Later specimens yielded ratios typical of tuberculous meningitis. Obviously a diagnosis based on the findings in the first fluid obtained in the early course of the disease may be erroneous. This observation was recently published by Fremont-Smith and Ayer.[4] The glucose content in twenty-two fluids (95 per cent) ranged from 0 to 54 mg., the average being 29 mg. It is interesting to note that the range for chlorides is below 700 mg.

Table II shows the results obtained in fifteen spinal fluids from ten cases of purulent meningitis. Four of these fluids (26 per cent) yielded false reactions in the Levinson test. Seven fluids (46 per cent) gave a heavier precipitate with acid, the average ratio being 1:2.6. The glucose content in Cases 2 and 6 in the first fluid was misleading, but in later specimens it was typical of purulent meningitis. In Case 8 the glucose value of 65 mg. was not typical. The chlorides were variable.

TABLE III

SPINAL FLUID OBSERVATIONS IN ASEPTIC MENINGITIS*

CASE	HEIGHT IN MM.				RATIO	GLUCOSE, MG.	CHLORIDES, MG.	CELL COUNT	GLOBULIN, GRADE
	SUL. 3%	$HGCl_2$ 1%	SUL. 6%	$HGCl_2$ 2%					
1	4	7	0.4	1.5	1:1.7	60	725	2200	2+
2	9	3			3:1	25	660	2800	4+
3	4	8			1:2	52		3170	4+
4	3	0	2	8	3:0	41	665	50	4+
5						57	690	7300	4+
5			5	10	1:2	58	745	4980	4+
6	8	15	11	26	1:1.8	13	675	7200	4+
6	10	20	11	31	1:2	45	645	6800	4+
6	15	4	25	11	3.7:1	48	665		
6	15	3	13	22	5:1	83	675	14000	

*Smears, cultures, and guinea pig inoculations were negative in each case.

Table III shows the observations in ten spinal fluids from six cases of aseptic meningitis. The Levinson test was done in the case of nine of these fluids, three of which showed false reactions. In only three was the 1:3 ratio evident which Levinson reports to have obtained in nontuberculous cases. The cell counts ranged from 50 to 14,000 for each cubic millimeter, and the glucose from 13 to 83 mg.; the average was 48 mg. The final diagnosis in these cases was dependent on the subsequent clinical course of the patients, the findings at necropsy, and the cavy inoculations. This is the group of cases most difficult to differentiate from tuberculous meningitis. The clinical symptoms, such as headache, vomiting, and even rigidity of the neck are common in both conditions, and the spinal fluid findings in this group are identical with those found in cases of tuberculous meningitis. It is only when all possible clinical data are correlated with the spinal fluid findings that a correct diagnosis can be established. The following report of a case will serve to illustrate the point:

A married woman, aged nineteen, came to hospital complaining of marked frontal headache, nausea, vomiting, and dizziness, of one week's duration. Since childhood there had been a discharge from the ear. Physical examina-

TABLE IV

SPINAL FLUID OBSERVATIONS IN SYPHILIS OF THE CENTRAL NERVOUS SYSTEM

CASE	HEIGHT IN MM.				RATIO	GLUCOSE, MG.	CHLORIDES, MG.	CELL COUNT	GLOBULIN, GRADE	WASSERMANN REACTION
	SUL. 3%	$HGCl_2$ 1%	SUL. 6%	HGCL 2%						
1	3	6			1:2	63	735	30	3+	4
2	7	6	5	4	1.1:1	83	725	0	0	4
3	2	4	2	4	1:2	56	701	38	2+	4
4	4	6	3	5	1:1.5	62	730	7	2+	4
4	4	10			1:2.5	58	755	60	3+	
5	4	8			1:2	86	700	40	2+	4
6	1	1			1:1	64	760	0	0	3
7	3	7			1:2.3	78	780	60	4+	4
8			2	4	1:2	81	745	8	0	1
9	3	4			1:1.3	73	785	6	1+	4
10			3	8	1:2.6	67	790	180	3+	4
11			4	8	1:2	60	753	85	2+	4
12	2	3			1:1.5	77	740	0	1+	4
13	1	3	1	2	1:3	62.5		60	3+	4
14	1	4			1:4	60		0	1+	–
15	1	4			1:4	70		10	0	4

TABLE V

SPINAL FLUID OBSERVATIONS IN VARIOUS CONDITIONS*

CLINICAL DIAGNOSIS	HEIGHT IN MM.				RATIO	GLUCOSE, MG.	CHLORIDES, MG.	CELL COUNT	GLOBULIN, GRADE
	SUL. 3%	$HGCl_2$ 1%	SUL. 6%	$HGCl_2$ 2%					
Meningismus	2	2			1:1	83	680	10	0
Meningismus	0	0			0:0	71	705	4	0
Meningismus	0	0			0:0	77	650	4	0
Meningismus	0	0	0	2	0:0	72		40	0
Meningismus	3	1	3	2	3:1	84	760	0	0
Meningismus			2	2	1:1	90		0	0
Psychoneurosis			1	3	1:3	72	800	4	1+
Hysteria			3	5	1:1.6	90		0	0
Fracture of skull	3	3	3	9	1:1	133	675	6	0
Fracture of skull	1	3			1:3	91	740	0	0
Fracture of skull	0	1	0	4	0:1	70	735	0	0
Neuritis	2	4			1:2	106		0	0
Neuritis	1	1	1	1	1:1	69	770	8	0
Apoplexy	2	2	2	2	1:1	68	720	4	1+
Glioma of brain	4	3			1.3:1	62	530	34	2+
Glioma of brain	3	10	2	9	1:3.3	76		0	0
Uremia	2	2			1:1	136		0	0
Encephalitis	1	1	5	4	1:1	75	760	10	0
Tetanus	1	1			1:1	77		3	0
Tetanus	3	4	2	6	1:1.3	83		0	0
Renal abscess	0	0	0	0	0:0	66	745	3	0
Headache	3	6	2	7	1:2	63	740	0	0
Pernicious anemia	3	7			1:2.3	56	700	0	0
Cholecystitis			3	5	1:1.6	71		0	0
Suspected lues	3	4			1:1.3	70		0	0
Suspected lues	2	0	2	0	2:0	79	740	0	2+
Suspected lues	1	2			1:2	70	710	0	0
Suspected lues	0	0	2	0	0:0	73		3	0
Suspected lues	2	5			1:2.5	62	770	4	0
Suspected lues	1	3	2	4	1:3	78		0	0
Suspected lues	1	1			1:1	84	750	0	0
Suspected lues	1	1			1:1	75		0	0
Suspected lues	1	2	1	3	1:2	81	700	8	0
Suspected lues	2	5	2	7	1:2.5	80		0	0

*Cultures and Wassermann reaction were negative in each case.

tion revealed a divergent squint and choked discs, more marked on the right; there was also a slight purulent discharge from the left ear but no evidence of mastoid blocking or edema. The leucocytes numbered 7,600, and the hemoglobin was 75 per cent; urinalysis was negative and her blood was sterile on culture. Lumbar puncture revealed a cloudy fluid: the cell count was 2,800, chiefly polymorphonuclears; globulin 4 +, and glucose 25 mg. for each 100 c.c. The Levinson test showed a precipitate of 9 mm. with mercuric chloride and 3 mm. with sulphosalicylic acid, a ratio of 3:1. No bacteria were found in stained smears of the sediment, and cultures in various media were sterile. A guinea pig was inoculated.

A roentgenogram of the head revealed digitation throughout the cranium. The pneumatic cells were obliterated on the left side.

In view of these observations the changes in the spinal fluid were considered secondary to brain abscess. Exploratory craniotomy revealed a cerebellar abscess; this was verified at necropsy, and later the cavy inoculation proved negative for bacilli of tuberculosis.

In Table IV are shown observations on sixteen spinal fluids from fifteen cases of syphilis of the central nervous system; fourteen (87 per cent) showed a higher precipitate with mercuric chloride. Of these, eleven (68 per cent) showed the 2:1 ratio considered pathognomonic of tuberculosis. The range of glucose content was within normal limits, from 53 to 86 mg., with an average of 68 mg. The chloride content ranged from 700 to 780 mg. for each 100 c.c.

Table V represents a group of thirty-four spinal fluids obtained in cases of various conditions in many of which the central nervous system may be indirectly involved: eleven (32 per cent) yielded false reactions in the Levinson test; sixteen (47 per cent) showed higher precipitate with mercuric chloride; three (8 per cent) higher precipitate with the acid; five (14 per cent) no precipitate, and ten (29 per cent) the same depth of precipitate with both mercuric chloride and sulphosalicylic acid. The glucose content ranged from 56 to 133 mg., an average of 74 mg. The chloride content ranged from 530 to 800 mg. for each 100 c.c.

SUMMARY

In the ninety-eight spinal fluids examined, 32 per cent yielded false reactions. In 100 per cent of the proved cases of tuberculous meningitis, there was a heavier precipitate with mercuric chloride, and in 90 per cent of these the ratio was 2:1 or higher. In the first specimens of spinal fluid, results in the glucose content as well as by the Levinson test were sometimes at variance with those obtained from later specimens. In conditions other than tuberculous meningitis the Levinson test is variable; this is particularly true in cases of syphilis of the central nervous system. Fluid showing a heavier precipitate with mercuric chloride, in the ratio of 2:1 or higher, may or may not be from a case of tuberculous meningitis. Fluid showing a heavier acid precipitate in our experience does exclude tuberculous meningitis.

CONCLUSIONS

1. The percentage of false reactions in the Levinson test is too high to be of positive diagnostic aid in tuberculous meningitis.

2. Low glucose values are not diagnostic of tuberculous meningitis, even in the absence of pyogenic organisms.

3. The glucose content of the cerebrospinal fluid yields valuable diagnostic information when correlated with all possible available clinical data.

The chloride content in tuberculous meningitis is less than 700 mg. and not constant in other conditions.

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LAMOTTE-WUTH BROMIDE COMPARATOR

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IN THE treatment of patients who are given bromides, either as a sedative or for epilepsy, it is of extreme importance that the dosage be so regulated that bromide intoxication be avoided. Such intoxication manifests itself either as deliria or as other mental or neurologic symptoms.

Careful work has shown that blood bromides are regulated in large part by salt (sodium chloride) intake; therefore, the dosage of bromide cannot be relied upon, unless it is studied in relation to the actual amount of bromides which reach the blood stream.

The LaMotte-Wuth Bromide Comparator was developed in cooperation with Dr. Otto Wuth, of the Henry Phipps Psychiatric Clinic, Johns Hopkins Hospital, using the method worked out by Wuth[1] for determining the actual bromide content of the blood serum. The test is so simply arranged that it requires very little technical skill and can be used as a routine laboratory test. At the same time it gives results that are sufficiently accurate for all clinical purposes.

The test is based on the fact that solutions of bromides give variable colors with gold chloride, the color depending on the bromide content. Before this test can be applied to blood serum, however, the serum must of course be coagulated and filtered. The test is then made on the clear filtrate, and the color secured is compared with standards representing definite amounts of sodium bromide.

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[1]Jour. Am. Med. Assn., 1927, lxxxviii, 2013.

The comparator, as shown in Fig. 1, contains 50 c.c. of trichloracetic acid reagent; 50 c.c. of gold chloride reagent; seven bromide color standards, representing 75, 100, 125, 150, 175, 200, and 300 milligrams of sodium bromide per 100 c.c. of serum; three test tubes; and two 0.4 c.c. pipettes fitted with nipples. These pipettes should be kept in the reagent bottles, since the nipples act as seals. Care should be taken that the trichloracetic acid reagent does not come in contact with the rubber nipple, as it will disintegrate it.

The top of the box contains three slots and is used as a comparator block in making measurements. A piece of etched glass is fastened to one side of the block, directly over the three slots, by means of a brass holder. This is a great aid in making accurate measurements, as it eliminates the reflection of outside objects in the tubes. The block should be held so that the etched glass is on the side facing the source of light.

A determination of the bromide content of the blood serum is carried out as follows: Draw 10 c.c. of blood from a vein, and allow it to clot. To 2 c.c. of the thus gained serum, add 4 c.c. of distilled water, then 1.2 c.c.

Fig. 1.

(3 x 0.4 c.c.) of Solution 1 (Trichloracetic acid). Shake, allow to stand thirty minutes, and filter. The filtrate must be clear; if it is not, filter again. To 2 c.c. of the filtrate, add 0.4 c.c. of Solution 2 (gold chloride), shake thoroughly, place in the comparator, and compare with the color standards. Comparison is best made by placing the test sample in the middle hole opposite the slots next to the ground glass plate, and then inserting color standards in the holes on either side, always making sure that the standards so placed are consecutive, that is 75 and 100, 100 and 125, 125 and 150, etc. If an exact match is obtained, the sodium bromide content is read off directly from the standard. If, however, the color of the sample lies between those of two consecutive standards, the intermediate value must be estimated. The numbers on the color standards give the sodium bromide content of the blood serum expressed in mg. per 100 c.c. A content of less than 100 mg. per 100 c.c. probably will be insufficient for epileptics. A content of over 150 mg. per 100 c.c. will denote an approach to bromide intoxication.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

CLINICAL AND EXPERIMENTAL

VARICOSE ULCER: Mechanism of Its Causation and Cure with Notes on the Associated Pain and Papillary Inequality, Byrne, J. Am. Jour. Med. Sc., October, 1926, clxxii, No. 4, p. 553.

The immediate primary mechanistic factor in varicose ulcer is incompetency of the superficial vein valves which, in the erect or sitting posture, permits the unsupported columns of blood to add their pressure to the normal intravenous pressure thereby counterbalancing or even overbalancing the intracapillary pressure; the net result being impairment of blood and lymph flow and increased tissue vulnerability.

Anything that impedes the return flow of blood to the heart from the lower extremities may act as a remote primary mechanistic factor by inducing blood and lymph stasis and dilatation of the veins.

Secondary mechanistic factors are: infection, cicatrized ulcer margin, enforced inactivity, endocrine dysfunction and impaired metabolism—general and local.

The fundamental indication in surgical or nonsurgical treatment is restoration of the local circulation by removal or support of the long columns of venous blood which impede or halt the capillary flow.

Many surgical and semisurgical methods of treatment of varicose ulcer are unsound in principle; excision and multiple ligation are sound in principle and applicable in certain cases, but they should always be supplemented by general and local prophylactic measures.

In severe, neglected cases treatment is best begun with recumbency and elevation which offset the immediate primary mechanistic factor.

In the majority of cases ambulatory treatment with compression bandages effects a rapid cure which may be kept permanent by prophylactic measures.

Study of the pain and pupil changes occurring in leg ulcer helps to a better appreciation of the sensory and pupil phenomena found in cardiac and other visceral disorders.

Endocrine treatment is indicated at one stage or another in almost every case of chronic ulcer, somatic or visceral.

Physical exercise is indicated as a cardiovascular tonic and as the best mobilizer and dispenser of the internal secretions.

ECZEMA, AND PROTEIN METABOLISM: Critical Investigation of the Relation of the End Products of Protein Metabolism to Eczema and Kindred Disorders, Michael, J. C. Arch. Dermat. and Syph., September, 1926, xiv, No. 3, p. 294.

Blood estimations of uric acid in eczema and kindred disorders are of doubtful value as evidence of a uratic pathogenesis of some cases of these diseases.

The improvement in eczema following institution of a purin-free diet can be construed in other ways than by its effect on the blood uric acid content.

Uric acid, urea and creatinine are not dermal irritants. In two persons, a moderate erythematous reaction was evoked, but only to concentrated solutions of uric acid and urea.

These metabolites have no demonstrable photosensitizing effects, or, at least, render the human skin mildly photosensitive only in a very small number of persons, and then only when in strong concentration.

Increasing the uric acid content of the blood by injections of lithium urate intravenously in two cases of severe, acute eczema produced no change in the subjective or objective symptoms of the disease.

MERCUROCHROME: Mercurochrome 220-Soluble in Malaria, Enbanos, F. Jour. Philippine Med. Assn., July, 1926, vi, No. 7, p. 215.

Report of the use of merurochrome intravenously in the treatment of malaria in seven lepers. The dose of mercurochrome was 0.003 gm. per kilogram continued in an average of 17 c.c. of 1 per cent solution, as a rule, only one injection being given.

In none of the cases were smears negative for parasites after the injection, though a marked symptomatic improvement (subsidence of temperature and chills) was noted in five cases.

ECZEMA AND URIC ACID: The Behavior of Injected Uric Acid in Patients with Eczema, Michael, J. C., and Nichols, H. O. Arch. Dermat. and Syph., September, 1926, xiv, No. 3, p. 308.

In a previous article, Michael reported a study of the relation of uric acid to eczema. In that article, two cases of eczema were reported in which intravenous injections of lithium urate were given. The purpose of the intravenous injection procedure was twofold: first, to determine the effect on the symptoms of the disease of artificially raising the blood uric acid content; and, second, to study the fate of injected uric acid in this disease. As disturbance of uric acid metabolism has been considered an important cause of eczema, it seemed worth while to determine whether patients with eczema actually showed any significant variation from the normal in their behavior to injected uric acid. The present report, therefore, is chiefly concerned with this biochemical phase of the question. In that respect it amplifies and supplements the previous report.

In the five cases here recorded, it has been shown definitely that artificially raising the blood uric acid level by intravenous injection of this metabolite has produced no enhancement of subjective or objective symptoms of eczema. As a matter of fact, in Case 5 this procedure was succeeded by definite improvement. In this instance the injection was followed by a chill and fever, and this may have been the important factor in the clinical improvement. Of course, other conditions such as rest and diet, may have entered the picture.

From the biochemical aspect, the blood uric acid data were in accord with the results obtained in normal persons by previous investigators. In every instance the injection was followed by an immediate rise in the blood uric acid level with a subsequent slow but steady fall, except in Case 5, in which this uniform decline did not occur. In this case the blood uric acid level may have been influenced by a febrile reaction which developed soon after the reaction.

The total recoveries of uric acid varied within moderate limits, as was to be expected. The recoveries are comparable to those of Folin and his collaborators for normal persons, except in Case 5, in which apparently none of the injected uric acid was eliminated. The elimination of uric acid is subject to considerable individual variation. The interpretation of the significance of total uric acid recovered following injection experiments is still a moot question. For these reasons no stress is laid on the uric acid recoveries in this small series. Indeed, Wells has made the point that urine recoveries of uric acid are valueless indexes of the fate of injected uric acid, since this metabolite may escape in unknown and unascertainable amounts through the intestinal wall.

Regarding the rate of elimination of uric acid after the injection the chart shows that the maximum rate occurs from one to three and a half hours after the injection, and that at no later period does the rate exceed the initial peak. There seems to be a gradual fall in the rate, and later increases probably are due to the ingestion of protein and carbohydrate foods.

Thus it is concluded from the data obtained from these patients with eczema that they differ in no essential way from the results in normal persons recorded by other investigators.

SCARLET FEVER IMMUNIZATION: Use of Sodium Ricinoleate Toxin (Larson) in the Immunization Against Scarlet Fever, Perkins, R. G., and Megrail, E. Ohio State Med. Jour., July, 1926, xxii, 599.

In a series of 1500 children of various ages, 361 or 24 per cent were found susceptible by the Dick test, using two test toxins checked against each other or known susceptibles and insusceptibles.

Among the 66 susceptible adults, 8 or 29 per cent showed a negative Dick test three to six weeks after a single dose of 3000 units of Larson toxin.

Among the 324 children available for retesting, 210 or 64 per cent gave a negative Dick test two to five weeks after 3000 units of Larson toxin, while 62 others showed faint or doubtful reactions.

If a negative Dick test is an indication of protection against scarlet fever, and since this reaction is very widely accepted as prima facie evidence, it appears that this small dosage will protect some 64 per cent or more, in a brief period.

Only one dose was used in this series in order to determine this point, but after retests this fall in the same series positives will be recommended to take at least two doses in the manner suggested by Larson. Another series is in progress, in which the positives have received two doses and will be retested this fall.

Adults were much more resistant to immunization than were children. The retests this fall will be followed by advice to the positives to take two or three doses as above.

The very small series of contacts in whom Larson toxin was inoculated as a preventive is of course without weight as itself, and valuable only as checking with the larger series of Larson.

If the immunity is shown to be of long duration, the absence of serious reaction and the lack of necessity for refrigeration of the toxin offer marked improvements in our activities against scarlet fever.

ARTHRITIS: The Classification and Treatment of Chronic Arthritis, Cecil, R. L., and Archer, B. H. Jour. Am. Med. Assn., Sept. 4, 1926, lxxxvii, 741.

In a study of 612 cases of chronic arthritis, approximately two-thirds (68 per cent) were of the proliferative type and one-third (30 per cent) were of the degenerative type.

Proliferative arthritis occurs most frequently in young people; degenerative arthritis is more often seen in the middle-aged and elderly.

The commonest form of proliferative arthritis is associated with focal infection about the teeth or tonsils.

The commonest variety of degenerative arthritis is the arthritis of the menopause.

The proliferative type of chronic arthritis is presumably an infectious process. Degenerative arthritis has not been proved to be of an infectious nature. It appears more likely that this form of arthritis, as the name implies, is a degenerative process analogous to arteriosclerosis and the other degenerative changes that attack various organs in middle age.

The essential feature in the treatment of proliferative arthritis is the removal of all foci of infection. In degenerative arthritis the treatment should be directed chiefly toward accelerating metabolism with iodides, physiotherapy, and other appropriate measures. Many of these patients are improved by a low caloric diet.

B. ACIDOPHILUS AND B. BULGARICUS: Viability of B. Acidophilus and B. Bulgaricus in the Human Intestine, Kulp, W. L. Jour. Am. Med. Assn., Sept. 11, 1926, lxxxvii, 833.

A report of studies from which it is concluded that B. Bulgaricus will not develop in or survive passage through the human intestine, and that, therefore, such preparations are without therapeutic value.

PNEUMONIA: The Epidemiology of Pneumonia, Powell, J. P., Atwater, R. M., and Felton, D. D. Am. Jour. Hyg., July, 1926, vi, No. 4, p. 570.

The purpose of this investigation has been to study the distribution of pneumococci among normal persons, the persistence of the organism in the respiratory tract, the virulence of the organism, and the existing relationships between the carrier state and season, age, sex and occupation.

This study covers 418 observations on 93 persons in four groups—high school boys, medical students, student nurses and laboratory workers—all living in the same city and during the same period.

There is good evidence that very few persons can long escape contact with some form of the pneumococcus under the conditions of our ordinary life. Moreover, contact with one of the fixed types of pneumococcus, forms associated with 80 per cent of all cases of pneumonia, is demonstrated for half the group during a period of seven months. Presumably, then, practically everyone at some time during the course of a year is a carrier of a fixed type pneumococcus.

Waves of unusual prevalence of the fixed types of pneumococci are described. These were not accompanied by corresponding cases of pneumonia in the same group. The figures for that year, in Boston, indicate that there was about one case of pneumonia for every 175 residents during the period of investigation. This group studied consisted of 93 persons, so that the fact that only one person developed the disease was within expected limits.

The observed prevalence of carriers of the different types of pneumococci, charted by months, suggests that we are dealing with organisms that go through seasonal variations in prevalence, variations which are probably different for the several types of the pneumococcus. The study of virulence by months also suggests that we are dealing with a biologic function of the parasite, analogous to well-known seasonal activities in the plant kingdom.

Study of the persistence of pneumococci in the respiratory tract of carriers shows that at least the fixed type organisms tend to disappear from the mouth and throat in less than thirty days. This was true in 37 instances out of 45 carriers who were found with these organisms. There was one instance in which a Type III pneumococcus was found in a person over a period of at least one hundred and twenty days, and there is reason, from virulence studies, to believe that this was persistence rather than reinfection. Three other cases showed persistence of from sixty to ninety days, but these were decidedly the exceptions. Type IV pneumococcus shows a persistence which is regarded as apparent rather than real, because of the variability in virulence of successively recovered strains and also because of the multiple opportunities for reinfection with indistinguishable strains of this type.

There appears to be no significant difference between the sexes in their tendency to become carriers of the pneumococcus. The four groups of persons show evidence that their mutual associations favor local contact and their pneumococci tend toward a uniform distribution of types within each group at one time.

When normal persons carry fixed types of pneumococci, they are rarely able to give any clue as to the source of these pathogenic varieties, a fact that has been brought out before in studies from this laboratory. These observations confirm the fact that persons in contact with cases of pneumonia more frequently carry fixed type pneumococci in their throats than controls, but carriers of fixed types are rather infrequent among the population at large. Persons may also get their pneumococci from contact with carriers. Therefore the isolation of cases of pneumonia could not be expected to be more than partially effective in controlling the disease.

Studies on the virulence of 216 strains, isolated during this investigation and tested on white mice, indicate that Types I and III tend toward a higher level of virulence than Types II or IV. In comparison with another study made in Boston and during the same year, there is observed an essential consistency of the virulence in all types recorded excepting Type II, in which an unusual number of avirulent strains was encountered.

The findings in this study, when compared with similar material from other sources, shows an essential consistency. In this composite fashion the data on carriers of pneumococci among 895 normal persons are given by types. Another comparison of data from 868

persons in Boston, all of whom were examined in this laboratory, shows the definite excess of carriers among normal persons exposed to cases of pneumonia.

In general these data serve to confirm the view that the disease pneumonia appears when there is a coincidence between infection with a pathogenic form of the pneumococcus and a lack of resistance in the same individual.

MEASLES: Skin Tests in Measles, Tunnicliff, R., and Taylor, R. E. Jour. Am. Med. Assn., Sept. 11, 1926, lxxxvii, 846.

The green-producing diplococcus associated with measles produces an extracellular toxin which gives a definite specific skin reaction in persons with a negative history of measles, but not in those with a positive history. The toxin is neutralized by convalescent human measles serum and by the serum of goats immunized with measles diplococci but not by normal goat serum.

These experiments indicate that antigens of killed measles diplococci (intracellular toxin) would be more effective for susceptibility tests than extracellular toxins on account of the former giving positive skin tests in about twice as many persons susceptible to measles as intracellular toxins (filtrates).

SYPHILIS: Syphilis of the Placenta in the Negro, McCord, J. R. Am. Jour. Obst. and Gynec., June, 1926, xi, 6.

Syphilis was demonstrated in 15.4 per cent of the 1,000 placentas examined.

In 966 women, the Wassermann was positive in 22.5 per cent.

In 655 babies, the cord Wassermann was positive in 0.7 per cent.

The Wassermann reaction repeated on 396 women agreed in 88 per cent.

Syphilitic placentas were found in 40.6 per cent of the positive Wassermann cases, and in 0.3 per cent of the negative Wassermann cases.

In 84 premature babies born alive, the placentas were positive in 28.5 per cent.

In 84 premature babies born dead, the placentas were positive in 67.8 per cent.

Of 219 positive maternal Wassermanns, the cord Wassermann was positive in 14.5 per cent of the cases.

Of 747 negative maternal Wassermanns, the cord Wassermann was positive in 0.107 per cent.

Of the positive placentas, 75 per cent had positive maternal Wassermanns.

FOREIGN BODIES IN THE LUNG: Pathologic Changes in Lung Tissue as the Result of Foreign Bodies of Long Sojourn, Manges, W. F. Jour. Am. Med. Assn., September 25, 1926, lxxxvii, 987.

For the purpose of this paper, a foreign body is one of long sojourn after it has been present in the air passages for two months or more and up to as many as thirty-five years, except that in one or two instances the sojourn has been less than two months but the pathologic changes are unusual. Serious pathologic changes do occur at times in cases of much shorter duration, but these are more or less constantly the acute type, such as infection, emphysema or atelectasis, with which physicians are quite familiar.

An aspirated foreign body in any portion of the tracheobronchial tree will sooner or later cause extensive permanent injury.

There is great variation as to the length of time a foreign body may be present before causing extensive changes, but those that interfere with drainage do, as a rule, cause injury early.

The permanent pathologic change is distal to the point at which drainage is blocked. The foreign body is at or distal to this point.

The end-results are in the nature of atelectasis, fibrosis, bronchiectasis and chronic abscess, with varying quantities of purulent exudate. Hemorrhage is common; tuberculosis is often suspected but is rarely present. The other lung remains remarkably free from pathologic change.

Manges believes that many of the one-sided, chronic, basal infections are the result of foreign body, regardless of history or of roentgenographic shadow of foreign body. Such lesions should at least be investigated bronchoscopically, and many should be treated in this manner.

FOREIGN BODIES IN AIR PASSAGES: Live Fishes Impacted in Food and Air Passages of Man, Gudger, E. W. Arch. Path. and Lab. Med., September, 1926, ii, 355.

Gudger, who is bibliographer to the American Museum of Natural History, has collected from the literature all the reports of impaction of live fishes in the human throat and air-passages.

The paper is most readable and of extreme interest but does not lend itself to abstraction.

LABORATORY TECHNIC

KOTTMAN REACTION: The Kottman Reaction in Dysthyroid, Neuropathic and Psychopathic Children, Mattei, G. Clin. Ped., May, 1926, viii, 28.

The author tested the Kottman reaction on seventy children with endocrine disturbances and various neuroses and psychoses and found that it showed great variability without any special uniformity in dysthyroid children while the reaction was almost constantly slight in idiots, cerebroplegics and epileptics (who had been given iodine treatment); this latter finding agrees with the original findings of Kottman.

As to the genesis of the reaction the author believes that the degree of viscosity of the blood plays an important part in it independently of the effect on the viscosity of changes in thyroid metabolism.

LYMPHATIC LEUCEMIA: The Neoplastic Nature of Lymphatic Leucemia and Its Relation to Lymphosarcoma, Evans, W. A., and Leucutia, T. Am. Jour. Roent. and Radium Therapy, June, 1926, xv, No. 6, p. 497.

Three cases of lymphosarcoma are described which in their terminal stages changed into lymphatic leucemia. These three cases are the only lymphosarcomata in a group of sixteen patients treated by deep roentgen-ray therapy during the last four years, which had a fatal termination. In all three cases radiation therapy produced prolongation of life with entire disappearance of the manifest lesions treated, but later extension to the bone marrow occurred. At the time of the bone marrow involvement, all three patients had developed the picture of lymphatic leucemia. Evidence is thus brought that lymphosarcoma, if life is sufficiently prolonged, may change into lymphatic leucemia. This happens as soon as the bone marrow involvement becomes the predominating feature of the disease.

The paper is accompanied by eighteen illustrations.

TYPHOID FEVER: Typhoid Vaccination by Mouth, Burke, V., and Barnes, La V. Jour. Infect. Dis., July, 1926, xxxix, No. 1, p. 67.

The work of several investigators indicates that certain bacteria introduced into the intestinal tract have antigenic action. Evidence of the antigenic action consists of positive agglutinin tests and increased resistance to infection. Evidence was obtained from both animal and human subjects.

The experiments described in this paper were designed to determine whether typhoid proteins were absorbed unaltered from the digestive tract as determined by the appearance of agglutinins; whether the agglutination titer would vary with the method of preparing the vaccine; whether "per os" vaccination is as effective as the subcutaneous method in stimulating the production of typhoid agglutinins; and to test the theory of increased cellular impermeability of the intestinal mucosa following the oral administration of typhoid vaccine.

The experiments were conducted on rabbits, the conclusions reached being as follows:

Agglutinins appear in the blood of some rabbits following introduction of standard typhoid vaccine by mouth. Artificial erosion of the intestinal mucosa in such rabbits is unnecessary.

Subcutaneous vaccination is more effective than vaccination by mouth in stimulating the production of typhoid agglutinins in rabbits.

The comparative degree of immunity to typhoid conferred by the two methods of vaccination remains to be determined. The comparative rate at which immunity develops, considered separately from the appearance of agglutinins, is also unknown. Since subcutaneous vaccination affords adequate protection against typhoid any superiority of the oral method, considered from the practical standpoint, is limited to simplicity of administration and reduction in toxicity.

The absorption of typhoid protein from the intestinal tract of rabbits apparently decreases upon continued exposure of the mucosa of the protein.

RHEUMATIC FEVER: Significance of the Leucocyte Count as an Index of Rheumatic Infection in Children, Wilson, M. G., and Kopel, M. Am. Jour. Dis. Child., July, 1926, xxxii, 46.

From a study of 384 counts on 88 children ranging in age from four to fifteen years, the conclusions following were drawn.

The average leucocyte count for children with a history of previous rheumatic infection (potential heart disease) was 7000 cells.

The average leucocyte count for children with organic heart disease (Class 1) was 7,700.

The average leucocyte counts for children with active rheumatic infection (carditis, arthritis, chorea and recurrent growing and joint pains) were above 9000 cells.

The correlation between vital capacity determination and leucocyte counts in children with rheumatic heart disease confirms the modern conception of cardiac failure in children being primarily due to infection rather than to mechanical factors.

Leucocyte counts within the normal range may be considered as indicative of the quiescence of the rheumatic infection, and counts above the normal range indicative of activity of the infection.

Repeated leucocyte counts in children with rheumatic infection would seem to be of some clinical value in diagnosis, prognosis and treatment.

PERNICIOUS ANEMIA: The Diagnostic Value of the Color of the Blood Serum in Pernicious Anemia, Fishberg, M. Am. Jour. Med. Sc., July, 1926, clxxii, No. 1, p. 81.

Fishberg notes that the color of the blood serum in pernicious anemia is always above normal and believes that this accounts for the characteristic color of the skin in this disease.

The serum pigmentation is generally due to an increase in the bilirubin content of the serum but not always, and may sometimes be due to hematin.

Fishberg concludes that:

In the active periods of pernicious anemia the blood serum is more highly pigmented than normally, being golden, golden-brown or rarely even dark brown. This is due to an increase in the bilirubin content and to the presence of hematin.

In an anemic patient the presence of pale blood serum speaks definitely against the diagnosis of pernicious anemia. Observation of the serum color is of particular value in the differential diagnosis between secondary anemia due to carcinoma and pernicious anemia.

MENINGITIS: Meningitis Caused by Bacilli of the Colon Group, Neal, J. B. Am. Jour. Med. Sc., November, 1926, clxxii, No. 5, p. 740.

Meningitis due to members of the colon group is comparatively rare. In studying over 1500 cases of meningitis only 7 were found due to these organisms. In an eighth case there were several organisms present, one of which belonged to this group.

Meningitis in the first three months of life is most often caused by the meningococcus —24 out of 50 cases being due to this organism and only 3 to the Bacillus coli.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Radiotherapy**

THIS little book is in no sense a reference book for the general practitioner; neither is it a short textbook. It is a general review on very broad lines of the subject of radiotherapy and its relation to the numerous branches of medicine. The fundamental principles of therapy are very well dealt with, and the effect of radiation on normal cells and diseased cells is well brought out. The attitude of the writer toward a single remedial measure is best explained by the closing paragraph of the book:

"Let us remember that nature recognizes neither the surgeon nor the radiologist, nor the expert in drugs, nor yet the lord of germs. She will put forth her best efforts only when she is assisted without stint. General practice is in reality the only logical form of practice. But no human mind can compass the whole field of medicine. Hence the hope of the future lies in specialism tempered by cooperation."

The Significance of the Physical Constitution in Mental Disease†

MEDICINE monographs are comprehensive reviews that adequately discuss a disease, certain aspects of a disease, or subjects that allow a better comprehension of disease processes.

In this, the tenth of this series of monographs, the authors have added an important contribution to the study of the relation of physical habitus to the reaction to mental disease.

In the early days of medical practice the family doctor was often credited with "knowing the constitution" of his patient and the trend of modern medicine which the reaction of the *individual* has led to interesting studies, as witness the contributions of Draper on the human constitution, of Dunn and Seegal on the correlation of facial form and disease and so on.

Wertheimer and Hesketh have undertaken an examination of mental patients from the standpoint of external habitus with the idea of testing the hypothesis that physical habitus and mental reaction types exhibit a more or less definite correlativity. In these studies both simple observation and exact measurement (anthropometry), are utilized and, as a result of the observations thus made a new anthropometric index is proposed.

Beginning with a review of early studies of body types, and of anthropometric studies in disease and psychiatry, the authors then compare the classifications which have been proposed in the past and discuss in general the methods used.

Their own studies were conducted on 65 male patients chosen at random who are classified into four groups:

1. Predominantly affective reaction types.
2. Predominantly schizophrenic reaction types.
3. Organic reaction types.
4. Psychopathic personalities and psychoneuroses.

*Radiotherapy in Relation to General Medicine. By Francis Hernaman-Johnson, Radiologist to the French Hospital, London. Cloth. Pp. 211. Oxford University Press.

†The Significance of the Physical Constitution in Mental Disease. By F. I. Wertheimer, Associate in Psychiatry, Johns Hopkins Hospital, and F. E. Hesketh, Carlton Fellow in Medicine, Johns Hopkins University, with a preface by L. F. Barker. Cloth. Pp. 76. 20 figures, 5 plates. Price $2.50. Williams & Wilkins Co., Baltimore.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

Data were assembled from observation and psychiatric and anthropometric studies from the analysis of which the authors suggest a new anthropometric index based on the formula:

$$\frac{\text{Leg length} \times 10^3}{\text{Transverse chest diameter} \times \text{sagittal chest diameter} \times \text{trunk height}} \times 100 = \text{index.}$$

The remainder of the book discusses the application of the index to the types studied. The contribution is of distinct interest to students of internal medicine and psychiatry.

*Collected Papers from the Henry Ford Hospital**

IN THIS volume, as the title indicates, are collected the scientific writings of the Staff of the Henry Ford Hospital from the date of its founding in 1915 until 1925. Although the period covered is of ten years' duration, most of the papers were written during the past two years.

After a foreword, there follows an alphabetical list of former staff members; a list of the present staff members, and then the three sections of the book proper: forty-three papers on varied subjects; an Appendix A, concerned with technical appliances; and the concluding section, Appendix B, which is a concise account of the history of the hospital and of its physical equipment.

The papers are arranged in alphabetical order according to their authors. An arrangement or grouping in accordance with their subjects,—surgical, medical, laboratory, etc.,—would seem preferable.

The subject matter is divided as follows: papers concerned with the investigation and clinical application of blood chemistry, 7; internal medicine, 5; surgery, 12; roentgenology, 3; obstetrics, 6; laboratory investigations, 10; internal medicine, 5.

The volume is an excellent example of the printer's art and is of interest both to those interested in the hospital and as a source of convenient reference on the subjects covered.

Klinisches Lehrbuch der Inkretologie und Inkretotherapie†

THIS is a four hundred page book which attempts to give a cross section of the subject of the internal secretions in sufficient detail for the man doing clinical work. One-third of the book is taken up with the structure and function of the glands of internal secretion, then follows a chapter on diagnosis and one giving rather complete clinical pictures of typical cases. Finally there are chapters on the significance of the internal secretions in the various fields of medicine and these chapters are written by various authors; that on internal medicine is by von den Velden, that on psychiatry and neurology by Rosenfeld, pediatrics by Schiff, gynecology and obstetrics by Aschner, urology by Bachrach, ophthalmology by Szily and Poos, otolaryngology by Kobrak, and dermatology by Rothman.

There are short bibliographies at the end of each chapter, but, as is so common in German books since the war, the references to American literature are most noticeable by their absence. Abel is mentioned in the text in connection with the active principle of the hypophysis, Aldrich with adrenalin, Banting and Best with insulin, and Kendall with thyroxin, but in no case is there a reference to the time or place of their publications.

However, the book gives in compact form the German point of view and so helpfully supplements some of our American texts.

*Collected Papers by the Staff of the Henry Ford Hospital (First Series, 1915-1925). Cloth. Pp. 634. 151 illustrations, 42 charts. Price $8.00. Paul B. Hoeber, Inc.

†Klinisches Lehrbuch der Inkretologie und Inkretotherapie. By Dr. Gustav Bayer and Dr. R. von den Velden. Georg Thieme, 1927.

*Preventive Medicine and Hygiene**

THE predominant function of the modern physician is the prevention of disease, and for an understanding of the principles underlying and applied to disease prevention a working knowledge of many and varied subjects is required. In this book, practically the only one of its kind, Rosenau has collected and presented in a most practical and utilizable manner, a vast amount of information of value to practicing physician as well as to the epidemiologist or the sanitary engineer.

This, the fifth edition of this well-known work, has been largely rewritten and reset and has undergone extensive additions and revisions. The book in general has two main divisions: (a) dealing with personal hygiene, and (b) dealing with environment (sanitation).

The material presented in the sixteen chapters of this book constitutes a veritable encyclopedia of methods applicable to the prevention of disease evaluated by an authority of extensive experience and presented in a most interesting and clear-cut manner.

New subjects which are considered comprehensively for the first time in this edition embrace: the psychoanalytic approach to sex hygiene, the prevention of cancer, the conservation of vision, periodic physical examinations, flukes and their relation to disease, granuloma inguinale, balantidial dysentery, resuscitation, gas masks, statistical methods, and practical points in public health administration. For some of these subjects Dr. Rosenau has enlisted the help of expert authorities.

The section on granuloma inguinale consists of but two short paragraphs which, in view of the increasing numbers of cases of this disease which are being reported, seems rather brief. The sections on statistical methods should enable many to grasp such methods to whom they are now an uncharted maze. Not only are all the important methods used in public health laboratories fully described, but numerous clinical procedures are also clearly detailed. There is practically no one, no matter how remotely interested in disease, for whom this volume will not prove an invaluable desk companion. It should be purchased for constant and daily use. It can be recommended without reserve.

The Inflammatory and Toxic Diseases of Bone†

AS STATED by the author, it is not easy to draw a line between the inflammatory and the toxic diseases as in both there are associated inflammatory changes, but a distinction may be made, however, between toxins locally produced by bacteria and circulating toxins from a distant source. The resulting conditions also differ, and the title of this book serves to indicate the distinction and the difference.

There are twenty chapters in which are discussed various bone lesions or diseases, namely, acute osteomyelitis, subacute osteomyelitis, serous periostitis and osteomyelitis, tuberculous disease of bone, syphilitic disease of bone, Charcot's joints, syringomyelia, yaws, arthritis deformans, pulmonary osteoarthropathy and allied conditions, rickets, infantile scurvy, osteitis fibrosa, osteomalacia, osteitis deformans, leontiasis ossea, and osteogenesis imperfecta.

Practically all of the illustrations are photomicrographs of gross or microscopic sections. These are excellently well done and greatly add to the value of the book.

The book amply reflects the experience of the author and is clearly and well written. There are numerous illustrative case reports some of which, however, are so brief and bald as to add but little to the text.

The author has drawn freely, and with profit, upon the collections of various English museums with the result that for each subject touched upon there are either gross or microscopic examples illustrated.

While not intended to be an encyclopedic discussion of the subject and, to some extent,

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*Preventive Medicine and Hygiene. By M. J. Rosenau, Professor of Preventive Medicine and Hygiene, Harvard Medical School. Cloth. Pp. 1458, with 157 illustrations. Fifth edition D. Appleton & Co., New York.

†The Inflammatory and Toxic Diseases of Bone. By R. Lawford Knaggs, Consulting Surgeon to the Leeds General Infirmary, etc. Cloth. Pp. 416, with 197 illustrations. William Wood and Co., New York.

reflecting the personal views of the author, the book well deserves a place upon the shelves of the student, the physician, the surgeon, and the pathologist as a valuable source of reference.

The physical craftsmanship of the publishers is most excellent.

X-Ray Therapy•

THIS little book of x-ray therapy consists of 120 pages. It sets forth the method of treatment as practiced in Professor Holzknecht's Clinic in Vienna. A short section deals very closely with general considerations of treatment. It is well worth reading, even by those not practicing x-ray therapy.

The greater part of the book deals with treatment of about seventy-five various diseases. Most of these are conditions in which the x-ray has proved of definite value. A very few are still in the experimental stage. Each disease is considered in the following manner:

Prognosis—a. Result of treatment
b. Duration of treatment
c. Course of disease under influence of x-ray

Accompanying and subsequent effects—
Adjuvant treatment
Contraindications
Treatment formula
Fields

The treatment formula is clearly set down; it is given in Holzknecht's units of course.

The book is cleverly compiled and makes x-ray therapy seem quite easy. It is intended as a guide for the man in general practice in deciding which cases are amenable to treatment, and a book of formulas for the radiologist. It serves both purposes well, but perhaps not as thoroughly as if they were dealt with separately.

Bacteriologic Atlas†

THOSE interested in bacteriology will recall the publication, twenty-five years ago of the *Atlas of Bacteriology* by Lehmann and Neumann of which the atlas here reviewed is reminiscent.

An illustration which *illustrates* is more rapidly, and sometimes more definitely, informative than several descriptive paragraphs, whose interpretation depends to no small extent upon the imaginative acuity of the reader.

The purpose of this atlas is to convey a picture of the various microorganisms illustrated as seen with the microscope under a magnification of about 1000 to 1500 diameters. The illustrations are drawn from tissue sections and from film-stained preparations and are accompanied by a brief description of the main features illustrated.

There are sixty colored plates, beautifully prepared and beautifully reproduced, all representing actual specimens from the author's collection. In a few instances (amebae, malarial plasmodia) forms from various parts of the slide are represented as appearing in one field.

For teaching purposes this should prove a very useful and valuable little book.

The illustrations are very well done, and the color reproductions are remarkably faithful.

•Index and Handbook of X-Ray Therapy. By Dr. R. Lenk, Privat-Docent of Medical Roentgenology, University of Vienna, with a foreword by Professor Holzknecht. Translated by T. I. Candy, Hon. Radiologist, Royal Ghent Hospital. Cloth. Pp. 120. Oxford University Press.

†Bacteriologic Atlas. By Richard Muir, Demonstrator of Pathologic Methods, University of Edinburgh. Cloth. Pp. 134. Price $4.50. Wm. Wood & Co., New York.

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EDITORIALS

Histories

THE foundation of many a reputation was laid when its possessor was an interne taking histories.

To many residents, history taking is a bugbear and a nuisance, often because of failure to realize that the hospital history is a de luxe edition of the stories to be elicited later in the office.

Every patient has a story to tell, sometimes without knowing it, and the development of the tale in its entirety very often depends almost solely upon the ability of the interrogator. As has been said, he must combine with the shrewdness and pertinacity of a prosecuting attorney, the finesse and tact of a diplomat.

Not infrequently a great mass of more or less irrelevant and nonessential details must be elicited and sifted to obtain the kernel of the story which may then be expressed in a sentence.

The taking of a good history is an art and a revelation, at the same time, of the skill and general knowledge of its compiler.

Each patient, in effect, subjects his physician to a general and searching examination as to his knowledge of the entire field of medicine. Very few patients will be prepared to recite a classical, textbook description of their complaint.

It is not only what they reveal but what they wilfully or unwittingly conceal which is often of paramount importance and which must be elicited by devious and tactful methods.

Pathognomonic symptoms are the exception. Most often it is suggestive "leads" unexpectedly discovered or suspected and unearthed which, when elaborated and dovetailed with the other data, lead to the ultimate clearing up of obscure conditions.

No part of the body is functionally a distinct entity from all the other parts and detection of a cardiac lesion or a renal deficiency should not be the termination of the examination but rather the starting point of an endeavor to correlate this finding with the rest of the human machine.

Thoroughness first, last, and all the time is the keystone of success.

Histories have other uses besides their application to the present case. Every good physician is a continual student, and the mine of his past experience often holds nuggets not to be found in books. Well taken and carefully kept histories more than repay an occasional rereading and study and bring to future cases the experience and information gained from past studies.

Moreover, the development of medicine depends upon the study and elucidation of past experience and it is, indirectly, from the compiled professional memoirs of physicians at large that the textbooks of the future will be written.

It is not enough to record good histories, they must be studied continually and utilized. Carefully taken, intelligently filed so as to be readily accessible, they form a permanent record of past experience and an accumulation of knowledge for future use.

Histories are compilations, condensations, and accumulations of answers to questions put by the examiner and the *meat of the answers may often be expressed in a sentence.* The value and significance of that sentence in suggesting a possible diagnosis depends as much upon the questions put as upon the answers received.

It is sometimes of little avail to ask a patient point-blank whether he has had a venereal disease or is a heavy drinker, for many patients are very Chinese in their endeavors, for reasons hard to fathom, to "save their face" and still others are supplying misinformation honestly.

An old Scotch doctor in charge of a dispensary in a section where the morning emesis and nausea of preprohibition days was comparatively common, was never known to ask the bulbous-nosed sufferer if he was a heavy drinker. He merely suggested that relief was sometimes obtained by a small drink of whiskey in the morning before breakfast; of course, the *first* one might not stay down, but the second would and gave comfort.

Who could refuse in the presence of such understanding to relate how the remedy was well known and often tried with the described result? Then,

when the patient had gone, with a quizzical look through his spectacles, the diagnosis would come: "alcoholic gastritis."

One must be on the alert in taking histories for unconscious hints as to further interrogations. Remember Cabot's simile of the cash register and devise your questions accordingly.

Investigation, correlation, and interpretation are the keystones of diagnosis.

—*R. A. K.*

Immunization Against Tuberculosis

THE hope of an efficient vaccination for tuberculosis has been revived in recent years by Calmette, who advocates the use of an attenuated organism of bovine origin, Bacillus Calmette Guerin (BCG).

The *Beiträge zur Klinik der Tuberkulose*, volume lxvii, contains an interesting symposium on artificial immunization against tuberculosis. Uhlenhuth calls attention to the need of finding out how nature at times succeeds in bringing about immunity to the tubercle bacillus, and recalls that immunity only exists when a focus of tubercle is present. The relative immunity of individuals depends on a more or less latent infection of childhood. The lowering of the vitality of the body, such as occurred in Germany during the year of hunger of the World War, allowed a spread of disease from latent foci.

The exact nature of immunity from focal infection is not known. Should a focus of tubercle completely heal, immunity is lost. Uhlenhuth relates experiments in immunization of cattle by injection of an old bovine culture isolated by v. Behring in 1902 from the mediastinal gland of a cow. The culture was only moderately virulent for guinea pigs, avirulent for rabbits, and almost entirely avirulent for cattle. The results of these experiments as tested by artificial and natural infection were completely negative. In view of such findings Uhlenhuth expresses surprise that Calmette with a somewhat similar culture should have succeeded in immunizing cattle. He critizes Calmette's experiments in that droplet infection plays the principal rôle in producing disease in cattle, and he believes the experimental cattle of Calmette were protected from this by their positions in the stalls. Evidence is produced from other workers which indicates that the BCG of Calmette can produce tubercles and that these may even at times go on to caseation. He agrees, however, that BCG is practically harmless and that years would be necessary to show that it might regain its virulence in the human or animal body. Avirulent cultures are useless for immunization purposes, as shown by Romer, Neufeld, Kraus, Selter and others, and Uhlenhuth states that if Calmette's work is proved, we are in contact with an absolutely new fact in immunization against tuberculosis.

—*G. B. W.*